1996

Transmission and immune response studies of toxigenic Pasteurella multocida

Paul Luther Sundberg
Iowa State University

Transmission and immune response studies of toxigenic *Pasteurella multocida*

by

Paul Luther Sundberg

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Veterinary Microbiology (Preventive Medicine)
Major Professors: George W. Beran and Lawrence E. Evans

Iowa State University
Ames, Iowa
1996

UMI Number: 9712610

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of

Paul Luther Sundberg

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Co-Major Professor

Signature was redacted for privacy.

Co-Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I have had the good fortune to have been associated with some people that I am proud to call my mentors and friends. Dr. Earl Barnawell, at the University of Nebraska - Lincoln, gave me the opportunity to observe how a university professor teaches, inside and outside of the classroom and laboratory. Dr. Eldon Uhlenhopp gave his time and energy to help me learn how to give my practitioner experiences the thought and order needed to teach and do research. Dr. Lawrence Evans kept me interested in academia during practice and gave me the chance I needed to further my education and take advantage of new opportunities. Dr. George Beran guided me through my university experience, helping me focus on applied epidemiology.

My father, Donald Sundberg, a true educator, has always inspired me as an example of wisdom and honesty. The experiences he gave me on the farm gave me the confidence to pursue my interests. My mother, Faith Bergquist Sundberg, gave me a solid foundation for personal, professional, and spiritual growth and taught me the value of patience and perseverance.

My bride, friend, and partner, Deb, has given me more than I could ever return. Without her support and love my life would be incomplete and my veterinary and graduate education would not have been possible. She has sacrificed her own professional career to give me the chance to advance mine. Her wonderful gifts of humor, sensitivity, insight and

intuition are unmatched. She has my heart and my respect and the respect of all her professional and personal acquaintances.

She gave us our daughters, Kristin, Kari, Lora, and Molly. Their energy and enthusiasm are our greatest pleasures. Each of them has such wonderful potential that is limited only by their imagination. They keep me going. They are the true meaning of *carpe diem.*

I. GENERAL INTRODUCTION

Dissertation Organization

This dissertation consists of a literature review, three papers, a general summary, and an appendix. Paper three is to be submitted to the American Society for Microbiology publication, Journal of Clinical Microbiology, and is written in the appropriate format. Papers one and two are to be submitted to the American Society for Microbiology publication, Infection and Immunity, and are written in the corresponding format. The appendix contains data from two of the individual projects, Tables 1 through 18 from paper 1 (Chapter III) and Tables 19 through 31 from paper 2 (Chapter IV).

Introduction

Livestock producers have three major options in dealing with bacterial diseases affecting their herds: antibiotic therapy, vaccination and herd management practices. Antimicrobial drugs are used as therapies in outbreaks of bacterial diseases, as disease preventives and as growth promotants. Vaccination against disease causing organisms attempts to raise the herd immunity level to prevent infection, minimize clinical disease signs, and avert economic loss. All in - all out production, medicated early weaning and segregated early weaning are management procedures designed to reduce pathogenic organism transmission. In practice, an acceptable combination of antimicrobials, vaccination and management can be used to maximize herd health and production.

The common mucosal immune system is responsible for immune defense functions on body mucous membranes such as the respiratory, reproductive and alimentary tracts and the surface of the eye. This system is stimulated by a distinctive antigen processing and presentation mechanism within these areas.

Sufficient antibody titer, at or near respiratory mucosal surfaces, aids in preventing respiratory disease. Antibodies elicited by vaccination or exposure and distributed to body surfaces are important in protection against pathogens trying to colonize mucosal surfaces.

The objective of this project was to supply details important to defining the epidemiology of toxigenic *Pasteurella multocida* while exploring immune system enhancement as an aid in control. Class specific antibody titer in sera and on the nasal mucosa was measured to see if it would be affected by intramuscular injection of a toxigenic *P. multocida* combination bacterin/toxoid. The use of beta-hydroxy beta-methyl butyrate as an adjunct to vaccination was investigated. The local antibody response to respiratory infection and its usefulness as an indication of pathogen transmission among pigs was studied. Finally, restriction endonuclease digestion was used to confirm organism identification in transmission studies among cohort pigs.

II. LITERATURE REVIEW

Progressive Atrophic Rhinitis

Introduction

Atrophic rhinitis, as a clinical syndrome, was first described in pigs in 1830 (40). The condition has been attributed to many causes, both noninfectious and infectious. Due to the involvement of the nasal bony structures, atrophic rhinitis was once thought to be caused by a nutritional imbalance, specifically an inappropriate calcium to phosphorous ratio in swine feeds (23). Because of breed predilections for the condition, genetic influences have been proposed (158). Environmental conditions have been implicated when other causes could not be identified (10, 161). Studies reported in the 1940s attributed the disease to an infectious etiology (134) and this remains the focus of research today.

Definition

While recognizing the importance and contribution of adequate nutrition, sound genetic background and environmental effects in the development of atrophic rhinitis, current research on the syndrome has focused specifically on the role of toxigenic *Pasteurella multocida*. This bacterium causes a swine upper respiratory disease characterized, clinically, by shortening or distortion of the snout, sneezing, nasal discharge and epistaxis. Hypoplasia of the nasal turbinate bones (conchal atrophy) is the characteristic lesion (40). Petersen et al. suggested the term Progressive Atrophic Rhinitis (PAR) to describe the disease syndrome caused by capsular types A or D toxigenic *P. multocida*. Their proposal further defines PAR as a disease in which the resulting turbinate atrophy persists after the first weeks of life. Conversely, Non-progressive Atrophic Rhinitis, caused by *Bordetella bronchiseptica*, causes turbinate atrophy early in life, with lesions regressing over time as healing occurs (129, 149).

Discovery of clinical signs or identification of conchal atrophy during slaughter examination with isolation of toxigenic *P. multocida* supports a clinical diagnosis of PAR. Subclinical forms of PAR are possible. Without external physical PAR signs, the producer may not be aware of subclinical infections. In moderate to severe cases however, the occurrence of lesions has been associated with reduced growth rates (40).

When either turbinate atrophy is described but *P. multocida* is not recovered, or toxigenic *P. multocida* is recovered but turbinate atrophy is absent, a PAR diagnosis is questionable. In the first case, turbinate damage may have occurred due to a subsequently cleared infection. The Missouri Veterinary Diagnostic Laboratory reports recovering *P. multocida* from only 30% of submissions in which PAR lesions were found (163). The second case may be from an aborted or limited infection without enough time for PAR lesion to result.

Prevalence

PAR prevalence in the nation's swine population is primarily found by clinical observations or by examination of snouts at slaughter, followed by bacterial culture. It has been estimated that 5% of the sow population carries toxigenic *P. multocida* without previous history of the disease. In cases of a low level of clinical signs, 10% prevalence has been estimated (27). A retrospective study done at the Missouri State Veterinary Diagnostic Laboratory found atrophic rhinitis in 27% of the necropsies done over a year's time (163). Slaughter examinations have led to estimations of 70% of market age swine showing signs of turbinate atrophy (115). Wilson et al., who sampled 1425 pigs from 27 randomly selected herds during summer and winter slaughter periods, found 60% of the pigs affected with atrophic rhinitis (174).

It may be possible for a swine herd to be *P. multocida* negative or have a herd prevalence so low the organism is not recoverable. Nine percent of 102 Iowa herds cultured positive for the organism, but toxin production was not determined (75).

When *P. multocida* is isolated from pigs, nontoxigenic strains are found more commonly than toxigenic strains. Forty-four percent of 163 pigs from nine farrow to finish herds cultured positive for nontoxigenic *P. multocida*, while only 4% harbored the toxigenic strain (37). Nasal and tonsil swabs from 468 piglets, of 30 farms, yielded nontoxigenic *P. multocida* from 24.7% of the tonsils and 18% of the nasal swabs without toxigenic strains being recovered (15). The wide variation in toxigenic vs. nontoxigenic recovery rates has yet to be adequately explained.

PAR and Production

Introduction

Extensive data have been collected regarding relationships between PAR and production parameters such as average daily gain (ADG), PAR and pneumonic lesions, and pneumonic lesions and ADG. There are convincing, yet at times conflicting, data describing these relationships.

Evidence of a *P. multocida* effect on weight gain has been shown by Varga et al. (168). During antibiotic treatment based on sensitivity patterns of isolated *B. bronchiseptica* and *P. multocida* from pigs, treated animals showed a 19% improvement over control animals in feed conversion. This study also found commercial herds medicated with antibiotics as young pigs had a 15.3% feed efficiency improvement and a 16% ADG improvement were found when *P. multocida* was not culturable from the nasal cavity through the end of the 42 day treatment period.

Numerous experiments have directly supported the claim that lesions of PAR are significantly correlated with weight gain and/or feed efficiency during the growing and finishing periods (9, 10, 11, 12, 38, 170, 174). Other experiments have arrived at equivocal results, with negative correlations between PAR and ADG being significant in only sections of some studies (14, 120, 130).

There are also those who contend PAR lesions present at slaughter are not indicative of decreased production parameters. Straw et al. (158, 159), Martineau et al. (104) and Love et al. (100), found no correlation between severity of turbinate atrophy and growth rate.

Toxigenic P. multocida toxin

The specific cause of a negative correlation between PAR and feed efficiency or ADG is not yet well defined, although *P. multocida* toxin has been incriminated. Cheville et al. saw severe systemic effects including hepatic necrosis, degenerated enterocytes in the gut lumen, kidney necrosis, edema in alveolar walls and necrosis of capillaries at cartilage-bone junctions when only 0.1 mg of toxin was given subcutaneously to pigs (32). Toxigenic *P. multocida* growth on a single horse blood agar plate can produce 15 to 30 mg of toxin (115).

Finding generalized systemic lesions led to investigations of the role absorption of *P. multocida* toxin may play in affecting production parameters. Love et al. (100), Frymus et al. (61) and Chanter and Rutter (27) theorized the systemic effects of locally produced toxin associated with PAR might decrease feed intake, contributing to lower weight gains. A depression in growth rate may result from a general toxic influence of *P. multocida* toxin, rather than from turbinate atrophy.

Goshal et al. investigated the effect of *P. multocida* toxin on porcine growth hormone level and actions (67). Porcine growth hormone concentrations in the plasma of naturally infected PAR pigs and healthy pigs were found to be similar. Decreases in growth rate of pigs with PAR, they hypothesized, may be because *P. multocida* toxin affects the signal transduction pathway that is associated with the porcine growth hormone receptor complex.

Prostaglandin

Pedersen and Elling, in studying the pathogenesis of PAR, speculated on the role of prostaglandins in the disease (128). Endotoxin likely contributes to the pathogenic potential of *P. multocida* by activation of host defense systems, including the production and release of prostaglandins. Prostaglandin effects contribute to clinical signs and tissue damage. They also have negative effects on feed intake and feed conversion (53).

One attempt at proving this theory involved pretreatment of pigs with prostaglandin synthetase, a prostaglandin inhibitor. Pretreatment did not limit skin lesions or dermonecrosis following intradermal injection of *P. multocida* toxin, however it is practically impossible to reach pharmacological concentrations of prostaglandin synthetase in tissues without killing the animals (53)

Immunoregulators

Immunoregulators such as interleukins, tumor necrosis factor (TNF) and interferon are found in the brain and cerebrospinal fluid during acute and chronic pathologic processes, such as those occurring with PAR (138). Considered to be endogenous mediators of the inflammatory acute phase response, they may cause inappetence, fever and somnolence (95, 138). TNF, Interleukin-1 and alpha and gamma interferon have reduced food intake after parenteral administration in animals (95). Fantino and Wieteska showed a reduction in food intake in rats due to direct TNF action on the central nervous system (52).

PAR and pneumonia

With conflicting evidence regarding the correlation of turbinate pathology (snout scores) on daily gain, the question becomes whether turbinate atrophy is an inconsistent sign of some other condition that affects gain. One theory is that turbinate atrophy may be present when lung lesions develop, but it is the lung lesions that are responsible for decreased growth and gain. Cowart et al. examined at slaughter 163 pigs from nine herds and found a positive association between atrophic rhinitis scores and the development and extent of lung lesions (37). Straw estimated a 3.3% daily gain reduction for every 10% of the lung mass with pneumonic lesions (159). It would follow PAR, by decreasing the filtering capacity of the nasal passages, can affect the incidence and severity of pneumonia, which in turn can affect production and growth. Lack of correlation between PAR and growth may be due to management factors decreasing lung involvement, even with PAR present in the herd.

The relationships among rhinitis, pneumonic lesions and production can also be disputed, however. Various studies have contradicted the theory of a positive relationship between snout scores and lung lesion development (64, 153, 158, 159, 174). Low correlation coefficients between individual pig lung and snout scores show rhinitis playing only a minor role in susceptibility to pneumonic lesions. A key may be that each of these studies examined the relationship between snout scores and lung lesions in individual pigs. Wilson et al. examined 1,425 pigs from 27 different herds spanning seasonal marketing periods and found atrophic rhinitis and pneumonic lesions were poorly related in individual pigs. When the herd, rather than individuals were examined, however, there was a significantly positive correlation (174).

Economics of PAR

Although the direct effects PAR has on growth are the subject of considerable debate, there is little doubt in today's swine industry that it is an economically important disease. In Iowa, it is estimated $111.86 per 1000 swine per month (approximately $166,000.00 per month) has been spent on PAR prevention or treatment costs (85). This is a substantial resource allotment for a disease in which research on production parameter effects is not unified.

When estimating a 6.7% (115) to 16% (168) reduction in average daily gain in affected pigs, the total cost of this disease increases dramatically. A 7% reduction in average daily gain results in at least ten extra days on feed to reach marketable weight (115). Estimating an increased cost of $2.46 to produce one marketable hog, PAR costs the United States swine industry as much as $203 million per year (115).

Clinical PAR

Diagnosis

PAR diagnosis is achieved by demonstration of nasal turbinate atrophy with isolation of toxigenic *P. multocida*. Although it is possible to have turbinate lesions without isolation, and isolation without lesions, a definitive diagnosis of PAR requires meeting both criteria. Techniques used for sample collection, bacterial isolation and turbinate evaluation will affect diagnosis and are therefore important.

Sample collection The most common live animal sample collection method involves passing a swab deep into the nasal cavity to wipe the nasal turbinate. While inexpensive and easily performed in the field, there could be concern clinically relevant isolations may be missed because of limited sampling area.

Although not easily applicable in field situations, nasal lavage increases the likelihood of sampling the entire nasal cavity. A problem arises in deciding what fluid and recovery method to use. Twenty milliliters of phosphate buffered saline has been instilled in one nostril of dorsally recumbent pigs and collected from the other. This method was reportedly more sensitive for detecting infected pigs than nasal swabbing (120). The exact site of sampling was not addressed, however. No direct swabbing of the tonsils and oropharyngeal region was included and it is possible flushing in this way could have resulted in sampling the oropharyngeal region, besides the nasal cavity.

Sample handling Handling of the collected samples is also an important consideration. During a study designed to evaluate sampling and processing procedures, it was found direct selective media inoculation from cotton swabs the same day as collection was the most reliable culture recovery method. Placing the samples in transport media, before culturing on selective media or by mouse intraperitoneal injection, decreased the percent positive swabs (28).

Media The media used for culture should also be considered. Columbia blood agar base with neomycin and bacitracin added is more effective in *P. multocida* isolation than 4% sheep blood agar, when gross colony characteristics are used for preliminary identification (77, 78). *P. multocida* isolation has been enhanced by intraperitoneal injection of mice with fluid from nasal swab samples suspended in sterile saline (41). This technique, although very sensitive, is time consuming, raises the issue of animal welfare and cannot differentiate toxigenic from nontoxigenic strains. It is also possible nontoxigenic strains could flourish and overgrow toxigenic strains or cause death of the mouse before toxigenic strains reach recoverable numbers (27).

In one study, *P. multocida* was recovered intermittently during the first week after inoculation in pigs already *B. bronchiseptica* culture positive. Only one pig remained consistently culture positive during the six week test period, while *P. multocida* was recovered sporadically from the other exposed pigs (77). This type of irregular recovery pattern was also seen during a field study in which sequential nasal swabs were collected from pigs on 8 Iowa farrow to finish swine operations. Sampling was done at 1, 3, 6 and 12 weeks of age, on each of four pigs, in five different litters from the farms. Of 80 *P. multocida* isolations, 14 were toxigenic. All 14 came from three of the eight farms during various weeks of sampling (78).

Slaughter check Examination of the cut surface of the snout at slaughter (slaughter check) is the usual method for finding the gross lesions of PAR. Commonly, the slaughter check is also used to evaluate the lungs for presence or degree of pneumonia, along with other health related parameters.

Clinical PAR is confirmed by post mortem examination of a snout transverse section at the level of the first or second premolar tooth (44, 105). The greatest space, in millimeters, is measured between the ventral turbinate scroll and the ventral/lateral nasal bone. Consistently sectioning at this site allows interanimal and interherd comparisons, because a natural widening of the space surrounding turbinates occurs as they course through the nasal cavity.

Various scoring systems have been used to quantify turbinate atrophy. A numeric grading system that describes the degree of atrophy and takes into account deviation of the nasal septum, if present, is commonly used (14, 44).

More recently, photographing the sectioned snout, followed by computer analysis of the degree of atrophy, has been described (35, 44). The linear perimeter of the area occupied by the turbinate is measured, as is the linear perimeter of the inside border of the nostril. These values are compared by ratio, describing the relationship of the area occupied by the turbinate to the total area available. This computerized tomography technique is useful for diagnosing PAR and, while comparable to visual post mortem grading systems, is less subjective (87, 130). When correlating production parameters such as average daily gain with degree of PAR, the computerized tomography is more accurate than visual measurement (130).

Factors affecting slaughter checks Additional factors influencing the validity of the slaughter check to imply herd PAR status need to be considered. Because positive culture and gross lesions define PAR, the likelihood of a false positive from a slaughter check is low. A false negative conclusion, however, is possible if gross lesions aren't recognized or toxigenic *P. multocida* is not isolated.

Sample size Slaughter check sample size must be adequate to provide a satisfactory probability PAR can be detected, or prevalence estimated, within a herd. Sampling all the animals in the herd or group would establish both the presence and prevalence of PAR but, without a whole herd test, at least a statistically based sample size should be used.

The appropriate sample size is based on the probability level of a false negative result one is willing to allow and the actual or estimated prevalence of the disease within the population. The equation used for deciding the number of animals needed for detection of a disease is:

$$n = \frac{\log Pb}{\log (1 - P)}$$

This is where n = appropriate sample size needed to detect a condition within a herd of infinite population size; Pb = the probability of a false negative result (as a decimal); and P = actual or estimated prevalence of the condition within the entire population (155). As one is willing to accept greater risk of a false negative result, or the prevalence within the herd increases, the number of animals needed to be sampled for detection will decrease.

Sample size determining prevalence is different from sample size for detection. A sample size appropriate for a population of infinite size is calculated by:

$$N_{inf\ pop} = \frac{(P)(1 - P)(Z^2)}{d^2}$$

$N_{inf\ pop}$ is the sample number needed given a population of infinite number, when P = the estimated prevalence expressed as a decimal, Z = degree of confidence in the estimate (refer to Table 1) and d = the

Table 1. Values for setting sample size (155)

Degree of Confidence in Estimate	Value of Z
99%	2.58
95%	1.96
90%	1.64
80%	1.28
50%	0.674

maximum difference between the observed prevalence and the true prevalence one is willing to accept (expressed as a decimal).

A sample size to discover prevalence within a defined population size can then be found by the equation:

$$N_{fin\ pop} = \frac{N_{inf\ pop}}{1 + \frac{(N_{inf\ pop} - 1)}{N}}$$

where $N_{fin\ pop}$ = the number needed to sample given a specific population size, $N_{inf\ pop}$ = the number needed to sample given a population of infinite number and N = the number in the finite population (155). Without sound statistical methods erroneous assumptions regarding the herd PAR status may result.

Seasonal variation Seasonal variation of slaughter check results in an individual swine operation can occur. Higher PAR scores and depressed growth indicators have been associated with pigs farrowed in winter months and marketed in the summer (153, 154). Slaughter check interpretation is most accurate when seasonal effects are taken into consideration.

Production stage A caution regarding slaughter check results comes from Scheit's work in Indiana. In his analysis, lung lesion scores did not significantly correlate with growth indicators during any season (152). The lack of significant correlation between lung lesion scores and growth indicators could be explained if gross pneumonic lesions are able to resolve within a certain period (153). It would follow this may also explain a lack of correlation between PAR and lung lesion scores. Slaughter checks are an examination at one point in time and may not represent the herd status at different production stages.

<u>Serology</u> An adjunct to the direct diagnosis of PAR by positive culture and lesion detection is serological testing. Specific anti-*P. multocida* toxin antibody present in the sera of non-vaccinated pigs is evidence of at least exposure to a toxigenic *P. multocida*.

Enzyme linked immunoassay Previous serological tests were based on detection of antibodies to epitopes on or within the whole *P. multocida* cell. As such, these tests were confounded by lack of specificity due to cross reactions with bacteria that share common antigens. The inability to identify a toxigenic strain unique antigen meant seroconversion could have resulted from exposure to nontoxigenic strains or other closely related non-Pasteurella bacteria. Given the ubiquitous nature of Pasteurella species, seroconversion to shared antigens made specific serologic diagnosis impossible.

Foged et al. have developed an enzyme linked immunosorbant assay (ELISA) using specific monoclonal anti-*P. multocida* toxin antibodies. This ELISA detects presence of the dermonecrotic toxin produced specifically by toxigenic *P. multocida* (58, 170), solving the problem of serologic cross reactions with nontoxigenic strains and related bacteria. Modification of the ELISA procedure allows detection of anti-*P. multocida* toxin antibodies in serum or body secretions (57, 122). Thus, it is possible to show seroconversion to the toxin, implying exposure, even without isolation of the toxigenic organism.

Seroconversion Toxigenic *P. multocida* infection, if localized in the upper respiratory passages, inconsistently results in seroconversion (61, 147). Localization of the infection on the nasal passage surface may allow the toxin to exert its effects on the nasal conchae without systemic immune system exposure. Alternatively, Chanter and Rutter suggest the lack of seroconversion may be due to a deficiency in the nasal cavity immune mechanisms, possibly toxin induced, or poor toxin immunogenicity when in the presence of other antigens (27).

Control of PAR

Transmission

Once PAR was established as a condition caused by an infectious organism, the study of transmission became important in addressing control and eradication. Initially much of the work was centered on *B. bronchiseptica*. Recent emphasis has shifted to *P. multocida*, specifically toxigenic strains.

Because nontoxigenic strains are frequently isolated from clinically normal animals, it is thought *P. multocida* exists as a normal resident on the nasal mucosa of healthy pigs (118). Nontoxigenic and toxigenic strains have been isolated from grossly normal snout and lung samples at slaughter (79).

Nielsen et al. concluded intermittent success in recovering the organism from inoculated pigs meant the infection in the nasal cavity is probably short lived (120). Continued transmission between infected and noninfected pigs must then occur to maintain the organism within the population (15, 37, 118). Housing pigs from a PAR herd with pigs without history of the condition resulted in PAR and isolation of both toxigenic and nontoxigenic strains from both groups (8).

The age of susceptibility to transmission is an additional factor that must be addressed to fully understand the epidemiology of the organism. Horizontal transmission (pig to pig) is different from vertical transmission (sow to offspring). An understanding of the relative importance of the two is necessary to design effective intervention strategies.

It was once assumed infection in piglets occurred within the first weeks of life (145). However, moving gilts from a herd of origin with minimal PAR to an isolated site with optimal environmental conditions does not affect PAR prevalence in the offspring (10). Vertical transmission occurs even with ideal cleanliness and air quality. Direct nose to nose contact between the sow and her offspring apparently can lead to transmission (10).

Horizontal transmission was shown among pigs in a study by Nielsen et al. Toxigenic *P. multocida* was isolated from 8% of a population when the pigs were 77 days of age or younger. After 77 days of age, 19% of the population was positive for toxigenic strains in nasal cavity, tonsil or lung samples. Transmission had occurred after the pigs left an all-in-all-out facility in which they were housed between 42 and 77 days of age (120).

Capsular serotyping and endonuclease digestion are tools used to better define the period of transmission. Oropharyngeal sampling of sows and offspring, and repeating the offspring sampling at 8 to 12 weeks and again at slaughter, uncovered no vertical transmission in a continuous flow farrow to finish operation. Horizontal transmission occurred between weaning and slaughter age (178).

Uhlenhopp et al. showed vertical transmission in a naturally infected farrow-to-finish herd. Post-weaning horizontal transmission then increased the number of culture positive offspring (164).

In summary, vertical transmission occurs early in life but the primary opportunity for spread is horizontal, post-weaning during grouping and regrouping of pigs as they grow. Either sows or finishing pigs would be the most reliable animals to swab when seeking the organism in apparently uninfected herds (70).

Management

Elías' study of PAR found an increase in incidence could be attributed to either the introduction of new toxigenic strains or a breakdown in sound management practices (47). A PAR outbreak could be blamed on introduction of new toxigenic *P. multocida*, if new stock were added to the herd and there was a change in the capsular antigenic composition, virulence, or toxin production. Should the strains isolated during an outbreak not differ antigenically from previous isolates, but show an increase in virulence, management factors could be incriminated.

In clinical situations, the incidence of PAR is most probably due to an interaction among many factors. Gardner et al. found the introduction of more than 50 pigs into the herd, despite the number of sources from which they came, was a significant PAR risk factor. Not included as risk factors were lack of herd replacement quarantine, antibiotic treatment and herd size (63). Because many factors may be involved (Table 2), with

Table 2. Factors thought to contribute to PAR.

• Presence of other pathogens (71)
• Poor management conditions/hygiene (27, 71)
• Poor ventilation (27)
• Overstocking/overcrowding (27, 69, 71)
• Movement of pigs between groups (69, 71)
• High dust levels (69)
• Immunological factors (69)

their definition and contribution subject to interpretation, the interaction between the environment and the pig's health might explain conflicting results concerning the PAR effect on rate of gain (10).

Environment

Air quality is a factor that is consistently associated with PAR. Mean snout scores, and percentage of snouts with a score of three or greater, from 1,117 pigs and 12 farms were significantly correlated with total air bacteria counts, particle size and ammonia concentration (113). It is thought the mass or number of particles present as inspirable aerosols or a large number of viable bacteria may compromise the local defense mechanisms of the upper respiratory tract, helping colonization of *B. bronchiseptica* and *P. multocida* (142).

Opportunity for continued exposure is another factor that may contribute to PAR. Housing various age groups (sows, weaners, growers and finishers) in the same shed aids in bacterial transmission and exposure (63).

Seasonal effects highlight the relationship between the infectious and the environmental components leading to PAR. Schwartz and Williams examined slaughter pigs over a four year period and found consistently higher PAR scores in those pigs farrowed in the winter and marketed in the summer (154). In examining the effect on ADG, Scheit et al. found as snout lesions become more severe, ADG decreased, but only for those pigs grown during the winter season (153). Even though air quality parameters were not directly measured in these two studies, they provide circumstantial evidence of an interaction between environmental factors and production effects of PAR.

While recognizing the importance of ventilation, Thomsen et al. implicated intensive pig unit stocking as a factor resulting in infection spreading by aerosol (161). Although the bacterium quickly loses viability when aerosolized, under conditions of high humidity (more than or equal to 79% relative humidity) *P. multocida* remains infectious much longer (161). Overcrowding, especially during the winter season when ventilation may be poor, makes reaching a sustaining humidity level possible. Poor survival in other liquids suggests routes of transmission other than direct exposure are unlikely. Nose to nose contact or exposing tonsils by eating nasal mucus contaminated fomites are the most probable transmission routes.

Further support for environmental importance is provided by Wilson et al. When looking at herd data, rather than individual data, they found increased herd average lung scores and average snout scores were both correlated with decreased space per feeder pig (174).

Vaccination

Vaccination during various stages of production can help control PAR. It is important for the pork producer to remember vaccination is only one part of a PAR control program and, by itself, may fail if attention is not given to other management and environmental factors.

Chanter and Rutter contend there has been no fruitful effort at reducing colonization by enhancing antibacterial immunity (27). Baalsrud supported this view by vaccinating pigs with a *B. bronchiseptica* and *P. multocida* bacterin and failing to show a significant reduction in PAR scores or increase in ADG (7). Rutter used a toxoid preparation in a vaccination schedule without affecting colonization in gnotobiotic pigs (144).

<u>Toxoids</u> Conceding that vaccination with bacterins alone may not affect growth parameters or colonization, work by investigators in Europe emphasizes the importance of a vaccine toxoid component. From his work on the development of a recombinant *P. multocida* toxoid and the ELISA for detecting toxin production, Foged theorized toxoid vaccination

failure may be due to the method of toxoid production (17). Inconsistent vaccination effects result if the production process destroys important immunogenic epitopes and there is no consistent reproducible way to standardize antigenic vaccine load (36). The work of Diemen, et al., even questions the value of a toxoid, suggesting that in vivo protective immune response is not directed toward PMT but to an unidentified bacterial component (166).

By showing a reduction in the frequency and severity of turbinate lesions (18, 88, 127, 133) and successfully affecting production parameters and colonization (18, 121), a toxoid could be part of a successful vaccination program. Immunization of pregnant sows helps protective levels of antibodies to be passively transferred to their offspring. Measurable antitoxin titers persist up to eight weeks of age in the offspring (93) and antitoxin titer may be a crucial factor in protection against PAR (121).

Other management practices

Antibiotic use Other management practices controlling PAR may include antibiotic treatments. Oxytetracycline, given either alone (68) or at the time of administering commercial toxoids (130), has been successful in reducing PAR lesions.

Isolated site production Geiger et al. recently eradicated PAR from a persistently naturally infected herd showing clinical signs by using a three site production system (66). Table 3 outlines the pig movement and antibiotic protocol used in this investigation. Comparing the snout scores of the isolated and medicated treatment group with the control group left at Site I, the protocol was successful in eliminating PAR lesions. Toxigenic *P. multocida* was not recovered from the control pigs though they exhibited typical PAR lesions. This may, however, have been due to sampling too few control pigs to reliably detect infection.

Table 3. Protocol of isolated site PAR eradication (66).

Site I
Ceftiofur to sows prefarrowing. Ceftiofur to pigs at 1, 5 and 7 to 10 days of age. Wean and move to site II at 7 to 10 days of age.
Site II
Ceftiofur to pigs at 12-15 days of age (5 days post-weaning).
Site III
Arrive at 10-12 weeks of age. No further medication before slaughter.

Eradication

Once epidemiological questions such as method and age of transmission and prevalence within different age groups have been investigated, scientifically based programs can be formed to control or eradicate PAR (70). The Netherlands began an eradication program in 1958 (39). Initially voluntary, in 1980 the government began a concentrated educational and financial effort to help pork producers with problems and questions regarding medication, vaccination, housing, ventilation and management. Although not yet complete, the eradication program has been successful in increasing the percentage of herds producing PAR free piglets from 5% to 64%. Percentage of herds with severe rhinitis problems dropped from 46% to 5% (39). These successes are due to attention being paid to all facets of the swine operation during the program, underscoring the need to address a variety of management and herd health issues to control PAR.

Pasteurella multocida

Bacterial characteristics

Pasteurella multocida is a bacterium in the family Pasteurellaceae and was named for Louis Pasteur, in honor of his work with the bacteria in avian species. The type species for the genus is *P. multocida* as named by Rosenbusch and Merchant in 1939 (103).

Morphology *P. multocida* was first isolated and described by Toussaint in 1879 (141). It is a Gram-negative nonmotile nonsporogenous coccobacillus or short rod. Virulent strains are usually encapsulated (141). Bipolar staining is common, especially in preparations made from infected animal tissues (103).

Growth The bacterium is a facultative anaerobe (103) that grows best in air or air plus 5% CO_2 (141). Colonies incubated for 18-24 hours, aerobically, at 35-37° C on enriched media such as serum, blood, or dextrose starch agars are typically 1-3 mm in diameter. They are mucoid or smooth and infrequently rough. Colonies of capsulated cells display a yellowish-green, bluish-green or pearl-like iridescence (141).

Table 4 lists the growth characteristics and biochemical reactions commonly used to identify the species.

Differentiating isolates *P. multocida* has been the subject of extensive antigenic testing. Serologic typing has been used to study the epidemiology of the bacteria in many species (103). Carter, in 1955, identified by passive hemagglutination four capsular types, designated A, B, C and D (25). Type C was subsequently discarded and type E was added to describe antigenically distinct serotypes (103). Heddleston et al. used antisera produced in chickens and antigen from heated cells for a gel diffusion precipitin test to establish the existence of 16 distinct somatic or O group serovars (103, 141).

Recently, additional techniques have been used to help further differentiate isolates. Electrophoresis of cell envelope proteins and lipopolysaccharide has shown the patterns of these cell components are conserved between *P. multocida* isolates (101). Restriction endonuclease analysis (REA) can also differentiate *P. multocida* isolates. Restriction endonucleases *Hha*I and *Hpa*II have been used to study prevalence and transmission within and between swine herds (74, 82, 173, 177, 178). This technique has even provided evidence of a single source of

Table 4. Growth characteristics and biochemical reactions of *Pasteurella multocida* (73, 103)

Characteristic	**Result**
ß hemolysis on blood agar	–[a]
Growth on Mac Conkey's agar	–
Indole production	+[b]
Catalase activity	+
Oxidase activity (Kovac's)	[+][c]
Indole production	+
Urease activity	+
Gas from carbohydrates	–
Acid production	
glucose	+
lactose	–
mannitol	+
galactose	+
inositol	[–][d]

[a]all strains negative
[b]all strains positive
[c]most strains positive
[d]most strains negative

transmission into the swine herds of Australia (65). Using REA, isolates of like somatic and capsular types can be distinguished. This technique is important in obtaining increased colony identification accuracy.

Virulence attributes

P. multocida pathogenicity is dependent on many factors, including the presence of bacterial virulence properties. *P. multocida* virulence attributes are listed in Table 5.

Capsule The *P. multocida* capsule is inconsistently found, being more closely associated with pathogenic rather than nonpathogenic strains (84). The indirect hemagglutination test, forming the basis for capsular typing, recognizes specific capsule associated antigens. There is a relationship between colony morphology and the presence of these specific antigens. Capsular groups A and D colonies display a pearl-like iridescence in oblique transmitted light (141).

A capsule forms the outermost surface of *P. multocida* and, before specific antibody development, inhibits normal serum bacteriocidal and opsonic activity (84). Mucoid colonies, due to hyaluronic acid in the capsule, have been found in all capsular group A and a few capsular group D strains. A large hyaluronic acid capsule associated with the capsular type A strains interferes with alveolar macrophage phagocytosis (136). Possessing a greater affinity for adherence to lung cells (169) and cultured with frequency from pneumonic lungs (79, 136), capsular type A is considerably more important in causing pneumonic lesions than

Table 5. Virulence attributes of *P. multocida.* (119)

• capsular polysaccarides
• surface structures such as outer membrane
• lipopolysaccarides
• neuraminidase
• multocidin
• adhesion structures
• toxin

capsular type D (79). Capsular type D is generally considered the type most often associated with clinical PAR (77, 156) but toxin production has been described in capsular types A and D isolated from pig lung and nasal passages during clinical outbreaks (150, 169).

Outer membrane proteins Outer membrane proteins induce the synthesis of protective serum antibodies and are assumed to be important determinants for bacterial survival. Their exact biological functions, however, are not known (119).

Lipopolysaccharide endotoxin Lipopolysaccharide endotoxin contributes to the pathogenic potential of gram-negative bacteria such as *P. multocida* (53). Its activation of the host defense systems, ultimately resulting in clinical signs and tissue damage, contributes to the pathogenicity of disease (53). The lipopolysaccharide electrophoretic pattern is conserved between isolates (101) and further study into their role in colonization or PAR development has yet to be done.

Exposure to sub-MIC levels of antibiotics has been shown to alter the lipopolysaccharide profile of some toxigenic stains while not affecting the expression of structural proteins or toxin. This exposure also diminished the virulence and adherence to porcine tracheal rings of these strains (97).

Other virulence factors Other virulence factors awaiting additional investigation include multocidin, an iron containing compound isolated from some strains (80), neuraminidase (151) and elastase (176).

Pasteurella colonization may be mediated by cleavage of epithelial cell surface fibronectin by elastase, exposing fibronectin receptors (22). A capsular type A *P. multocida* isolated from a pneumonic swine lung colonized the tonsils of a human and produced clinical illness. Upon investigation, the site of the adherence was thought to be fibronectin receptors in the human tonsil (176). Although not yet investigated, there is the possibility of such a *P. multocida* receptor in swine.

Colonization Colonizing ability and toxin production are considered the most important *P. multocida* virulence factors (98). As early as 1982, Rutter and Rojos described the differences in isolate pathogenicity to be associated with the ability to colonize the nasal cavity or produce toxin (149).

Fimbriae and pili Toxigenic *P. multocida* associated with clinical PAR possesses fimbriae (73, 137, 162). Fimbriae, functioning as adhesins, play a role in the colonization and infection of the nasopharyngeal mucosa (73). If present on *P. multocida* infecting tracheal epithelial cells and the deeper lung tissue, fimbriae enhance neutrophil and macrophage phagocytosis (137).

The existence of pili is not without some question. iDali et al. (81) and Grund et al. (72) used transmission electron microscopy to look for pili on toxigenic *P. multocida*, Grund finding them on only two of 79 isolates. iDali found no evidence of pili or flagella. Pijoan and Trigo found different tissue preparation techniques affected the ability to show colonization. They discovered those prepared for light microscopy were consistently *P. multocida* negative, but their preparations for scanning electron microscopy showed both turbinate and tonsillar colonization. Because of this, they contend discrepancies in the reported presence of pili are artifactual and due to different fixation and processing techniques, causing delicate pili destruction before examination (137).

Pili are not an absolute prerequisite for *P. multocida* upper respiratory colony formation. Nonfimbriated toxigenic capsular type A cells, as well as fimbriated toxigenic capsular type D cells, can adhere to newborn pig turbinate explants (137).

Sites of infection Although attention has been focused on nasal turbinate infection, alternate infection sites can serve as a source of lesion producing toxin (3, 137). The tonsil may play a significant role in PAR pathogenesis, serving as a readily available site for colonization and as a location for absorbance of pathologic toxin concentrations (3, 5). Ackermann et al. found histological lesions from infection in the tonsil increased in severity over time without increase in colony forming units. Intranasal inoculation could result in ongoing tonsillar infection. Using light and electron microscopy, he found *P. multocida* free within tonsillar crypts and within macrophage and neutrophil phagosomes (3).

Some controversy regarding colonization in the nasal passage may result from experimental procedure. Chung et al., after incubating swine turbinate fragments with bacterial suspensions, concluded *P. multocida* capsular type A or D was incapable of adhering to ciliated epithelial cells (34). It has an affinity to respiratory tract mucus (98) so failure to show adherence to turbinate fragments may be due to the processing technique, washing from the tissue *in vivo* produced mucus that acts as a receptor site (137).

Tissue predilection is based on structural characteristics of the infecting strain. Jacques et al. found capsular type A could adhere in greater numbers to porcine tracheal epithelial cells than capsular type D (86). Other studies show an increased likelihood of isolating type A cells from deeper lung tissues (79, 136). Pijoan and Trigo explain site affinity by showing capsular type D is more likely to be fimbriated, allowing adherence to the ciliated nasal turbinate but attracting alveolar immune cells. They show a consistently thick hyaluronic acid capsule, without fimbriae, in capsular type A. Although being a disadvantage in the nasal passages, the thick capsule enhances colonizing capabilities in the lower lung by aiding in immune system avoidance (137).

Relationship with concurrent infections

Bordetella bronchiseptica *B. bronchiseptica* is a common inhabitant and pathogen of the pig respiratory tract, causing transient turbinate hypoplasia but no snout changes in young pigs (27). Many clinical and experimental studies have shown enhancement of infection and PAR development when *B. bronchiseptica* is present (76, 118, 125, 126, 149, 156), but toxigenic *P. multocida* distribution is more closely associated with PAR prevalence than is *B. bronchiseptica* (27, 156). When Söderlund, Norqvist and Thafvelin sampled PAR and PAR free herds, they found no difference in *B. bronchiseptica* isolation frequency between herd types. The frequency of toxigenic *P. multocida* isolation, however, was higher in those herds affected by PAR (156).

B. bronchiseptica appears to be able to colonize the upper respiratory tract without predisposing factors easier than P. *multocida* (48). After experimental inoculation, three times more *B. bronchiseptica* than *P. multocida* was found to adhere to swine nasal epithelial cells (118). *B. bronchiseptica* adherence and infection is probably dependent on the amount of *B. bronchiseptica* specific secreted IgA antibodies present in the upper respiratory tract (48).

The level of *B. bronchiseptica* infection then influences initial colonization efforts of *P. multocida*. It produces different low molecular weight, heat stable substances that alter the nasal cavity conditions to favor *P. multocida* colonization (45, 112). These substances most likely include hemolysin, adenylate cyclase, an adhesin and a tracheal cytotoxin (26, 27). Infection and tracheal cytotoxin causes ciliostasis, cilia depletion, lymphocyte and neutrophil submucosal infiltration, and mucus accumulation on the tracheal and nasal epithelium (45, 118, 140). Mucus accumulation and ciliostasis are thought to be principal factors allowing *P. multocida* colonization.

Viral infections Intriguing is the possible relationship between viral respiratory infections and *P. multocida* colonization. Briggs and Frank found increased elastase activity in nasal secretions of calves exposed to Infectious Bovine Rhinotracheitis, a herpes virus. Quantitatively assaying for nasal secretion elastase and *Pasteurella hemolytica* concentration, they showed increased elastase activity preceding colonization and decreased activity before the concentration declined (22). They hypothesized viral infection contributes to enhanced bacterial colonization by reducing ciliary clearance, altering bacterial nutritional status, altering immune factors and/or exposing or altering epithelial surface cell receptors. Elastase has been implicated as the cause of fibronectin receptor exposure in one case of human tonsillar colonization (176). Herpes viruses endemic in some swine populations, such as pseudorabies virus or cytomegalovirus, have yet to be studied regarding this type of association with colonization.

Piracy of adhesins While affinity differences between capsular type A and capsular type D have not been found, *P. multocida* has shown an affinity for respiratory tract mucus (98). A phenomenon termed "piracy of adhesins" may explain the mucus attraction. Filamentous hemagglutinin and pertussis toxin, mediating respiratory cilia adherence, is secreted by human *B. pertussis* . These secreted adhesins will also bind and mediate respiratory attachment of *Streptococcus pneumoniae*, *Hemophilus influenza* and *Staphylococcus aureus*. This may also occur in pigs, involving the adhesins secreted by *B. bronchispetica* and explaining *P. multocida* adherence to porcine mucus (98).

Experimental Infection and Recovery

Experimental model

Experimental models developed for PAR study are based on the relationship between nasal epithelial irritation or *B. bronchiseptica* infection and *P. multocida* colonization. Intranasal inoculation after instillation of dilute acetic acid, is one method used to initiate colonization (49, 128, 165). Acetic acid produces subtle biochemical changes without overt nasal epithelial damage. These changes are temporary compared to those caused by *B. bronchiseptica* (27).

Ackermann et al. developed an experimental model based on the *B.*

bronchiseptica - *P. multocida* relationship. Intranasal exposure to a sterile toxigenic *B. bronchiseptica* sonicate, followed by intranasal instillation of toxigenic *P. multocida*, produced typical PAR turbinate atrophy (6).

Whether noninfectious environmental conditions can cause changes in the nasal cavity that enhance colonization remain to be investigated. Concentrations of ammonia, dust and other pollutants in an enclosed facility's air change with seasons and ventilation rates. If irritants are present in sufficient concentrations, acetic acid-like changes to the nasal epithelium, predisposing to infection, may occur. This could be one factor explaining increased PAR prevalence in pigs grown during the winter months (153, 154, 161).

Recovery of the organism

Recovery of experimentally or naturally infecting *P. multocida* is inconsistent. Commonly, in an exposed pig, the organism is recoverable at intermittent times during a study period. Voets et al. recovered an inoculated *P. multocida* from 79% of directly challenged pigs (170). In another study, at 2, 4 and 6 weeks post inoculation 40, 40 and 65 percent, respectively, of inoculated pigs were culture positive (170). Hoffman reported a similar situation after experimental inoculation into pigs naturally infected with *B. bronchiseptica*. Pigs were *P. multocida* culture positive within the first week post challenge and periodically during the study. Only one pig remained culture positive throughout the six week testing period (78). Similar intermittent recovery success from pigs naturally infected has been reported (77, 110).

Pasteurella multocida Toxin

Introduction

The toxin produced by *P. multocida* has been of interest since 1975, when the relationship between the toxin and PAR was first described (83). Support of this relationship is found in many investigations (59, 62, 83, 126, 144).

Adhesion was best achieved by toxigenic strains, without regard to fimbriae presence, hemagglutinin or capsular antigenic type, according to Pijoan and Trigo. (137). Chanter (26) and Chanter and Rutter (29) propose *P. multocida* toxin (PMT) is the main colonization factor produced by the bacteria, while Vena et al. found no correlation between adherence and PMT (169).

Many studies agree PMT is produced by either capsular type A or D (79, 136, 141, 169). PMT produced from either capsular type is serologically (60, 62) and biologically (50, 150) identical.

A similar toxin may also be produced during infection of species other than swine. A bovine isolate was found to produce a toxin with biological properties similar to PMT. This toxin also was similar to swine PMT on Western blot and in polyclonal and monoclonal antibody reaction (90).

Characteristics

PMT is a protein with a molecular weight of 143,000 Daltons on SDS-PAGE (1, 59) and 146,306 Daltons by sequence analysis (24). Trypsin treatment (cleaving peptide bonds) and sodium dodecyl sulfate or dithiothreitol treatment (breaking disulfide bridges holding polypeptide chains and other covalent bonds) yields three fragments (26, 94). The three fragments are not individually biologically active (94) but can reassociate into a fully active protein (26). Smith, et al., suggest the toxin is internalized by the cell, having an intracellular enzymatic action on an unidentified target molecule (171).

Genetic basis A toxigenic capsular type D *P. multocida* chromosomal DNA library in *E. coli* has been developed by Petersen and Foged (131) and Lax and Chanter (96). Two clones were identified by monoclonal antibody as producing PMT. Genetic mapping of the cloned regions identified the gene responsible for PMT production, and it was named toxA (131). Subsequently, a DNA probe including the toxA gene differentiated toxigenic from nontoxigenic strains of both capsular types A and D (60).

Petersen et al. produced multiple deletions in the toxA gene by restriction enzyme digestion. Four purified toxin derivatives, lacking different and widely separated regions in amino acid sequence, lacked toxic activity. One derivative produced efficient protection against challenge in mice and was the basis for a recombinant PAR vaccine (132).

<u>Production</u> PMT is first detected after 16 hours of growth following inoculation of 10^1 CFU/ml in broth culture, when the viable cell count reaches 10^8 CFU/ml (147). This corresponds to the late logarithmic to stationary growth phases (27, 147). PMT has not been detected in the supernatant of log growth phase cultures but is present in cell lysates of this phase (81).

PMT does not fit the classical definition of an exotoxin, as it is released only upon cell death. Scanning electron microscopy of specially stained cells showed PMT in the cell cytoplasm. It was not found on the surface or in the extracellular space of intact undamaged cells (81).

<u>*Biological effects*</u> PMT biological effects have been well studied, especially as they relate to the pathogenesis of PAR. Many species other than swine can be affected. In rabbits, pneumonia has been induced following iatrogenic administration (33) and a clinical condition similar to PAR has been reported to result from natural toxigenic strain infection (42). Turbinate atrophy (59) and death (56, 59) has occurred in rats and mice given PMT.

The sensitivity of BALB/c mice to cell free *P. multocida* sonicates has been reported to be up to six times greater than that of other mouse strains (144). Differences in sensitivity, and differences in PMT preparation purity, may explain reports of a 50% lethal dose (LD_{50}) in mice ranging from 0.5 to 15 μg/kg (56).

Systemic and local effects PMT has multiple systemic effects when administered to the pig. These effects may be fatal (32, 115), with an LD_{50} ranging from 50 ng/kg to 800 ng/kg depending on the weight of the pig, with lighter weights being most sensitive (56). PMT causes a general suppression of a pig's well-being, reducing the pig's activity, food intake and production of heat (167). When given by parenteral injection, PMT causes hepatic necrosis, enterocyte degeneration, kidney necrosis, alveolar wall edema, capillary necrosis at cartilage and bone junctions (148, 172), sinusoidal neutrophil infiltration and Kupffer cell hypertrophy (109). The toxin has no effect on alveolar macrophage activity. This supports the contention that toxigenic strains, primarily of capsular type D, are nonpathogenic in enzootic pneumonia (135).

The common and consistent lesion in affected tissues is vascular endothelial damage (32, 106). Devastating systemic effects, such as liver and kidney disfunctions, caused by PMT amounts produced by natural infection, suggests nasal turbinate lesions alone do not inhibit growth or affect weight gain (115). This may, instead, result from a general toxic influence (27, 61, 100, 165).

PMT given by intramuscular (105, 106, 109, 148), intraperitoneal (31, 148) or intravenous (148, 172) routes causes nasal turbinate atrophy consistent with PAR lesions developing from natural infection. Instilling PMT intranasally causes PAR lesions (59), unilateral intranasal exposure causing bilateral nasal turbinate atrophy (43).

PAR turbinate atrophy is related to bone resorption not compensated for by new bone formation (50, 107). It is generally accepted that PMT increases osteoclast number and activity within the turbinate matrix, while decreasing osteoblast number and activity (43, 50, 59, 106, 109), without histological evidence of inflammation (59). The increase in osteoclastic activity may be mediated by PMT stimulation of IL-6 release from fibroblasts (143). Work by Ackermann, et al., however questions the effect of osteoclasts by showing that even though there is rapid bone resorption in the concha, acid phosphatase expression and number of osteoclasts in the concha decrease after subcutaneous PMT administration (2).

PMT acts as a chronic stimulus on the whole skeleton, but its pathologic influence is seen in the highest bone turnover sites (49, 61). The turbinates are the fastest growing bone structure in the very young pig (49). Their bony turnover is so intensive the ventral nasal conchal scrolls are completely renewed within the first two weeks of life (108). As they continue to grow, the bony structure has been estimated to multiply 16 times within the first month of life (160). It is not surprising PMT given to young pigs can cause PAR lesions as quickly as four days postexposure (105).

Conflicting reports of PMT effects on other bone formation locations have yet to be resolved. After giving PMT by various parenteral routes, Rutter and Mackenzie found no histopathologic changes in pig humeral epiphysis or metaphysis sections or at costochondral junctions

(148). Yoshikawa and Hanada (175) and Ackermann, et al. (4), however, found histopathologic changes in the epiphyseal cartilage matrix throughout the body during spontaneous and experimentally induced PAR (175).

Role in PAR Pathogenesis

Development of PAR is a multistep process, each step being a prerequisite for the next. Local immunity and the normal indigenous flora act synergistically, maintaining a balanced bacterial flora in the upper respiratory tract (16). PAR begins when the nasal mucosal resistance is weakened by *B. bronchiseptica*, or other infectious or noninfectious factors. Toxigenic *P. multocida* then colonizes the nasal mucosa and toxin is produced. The toxin, either by local infiltration or systemic absorption, enhances osteoclastic resorption and impairs osteoblastic turbinate osseous core synthesis. These changes become irreversible within a few days. Nasal epithelium and submucosa undergo secondary atrophy and turbinates may disappear almost completely within 10-14 days (49).

Detection

Dermonecrosis Both PMT and *B. bronchispetica* toxin cause dermonecrosis when injected intradermally in the guinea pig (30, 51, 59). The two toxins are similar in some biological properties (56) but do not have mutual immunologic antigenicity (30). Similar dermonecrotic lesions resulting from intradermal injection of cell free toxigenic

Clostridium perfringens preparations support the nonspecific nature of PMT induced skin lesions (51).

PMT caused dermonecrosis allows differentiation between toxigenic and nontoxigenic *P. multocida.* Guinea pig intradermal injection of either sterile cell free filtrates or cultures have been widely used for PMT assay (41). The dermonecrotic test, however, is time consuming, expensive and may be considered unnecessarily cruel.

Mouse inoculation Mouse inoculation can be used to detect PMT. Intraperitoneal injection of toxigenic or nontoxigenic strains is lethal in mice. Toxigenicity is determined by death following intraperitoneal injection of sterile cell free filtrates. This diagnostic test is now used infrequently, due to animal welfare concerns and possible false positive results (41, 56).

Cytopathic effect PMT produces a cytopathic effect on Vero (African green monkey kidney) cell (56) and embryonic bovine lung cell monolayers (146). The embryonic bovine lung (EBL) test is the preferred cytopathic assay. It is more sensitive and reliable than Vero cell assay (56) and is 10^3 to 10^4 times more sensitive than is lethal assay in BALB/c mice (147). Detection is reported at 30 to 179 pg levels (54, 56, 59, 117). An agar overlay technique, using EBL cells, can also be used as an *in vitro* PMT assay (30, 46).

Monoclonal antibody detection An enzyme linked immunosorbant assay (ELISA) to detect PMT has been developed (58).

Coating specific anti-PMT monoclonal antibodies onto microtiter wells allows detection of toxigenic strains from mixed swine nasal or oropharyngeal cultures (19, 170).

The sensitivity and specificity of an ELISA using bacterial antigens depend on the specificity and quality of the antigen preparations (122). Appropriate capsular, somatic or pilar *P. multocida* antigens that give satisfactory ELISA specificity have not been identified.

PMT is exclusively produced by toxigenic *P. multocida* and can be used as evidence of its presence, even when the colonies are not grossly recognized in culture. Through separate studies involving 8000 nasal swabs from 215 Danish SPF herds (57), 615 field isolates, 47 *P. multocida* strains (including 13 confirmed toxigenic by dermonecrotic and cytopathic effects (169)) and seven reference strains (58), the ELISA specificity has been established as very near 100%. It has been approved for use in the Denmark PAR eradication program, establishing toxigenic strain presence in primary nasal cultures (56).

The ELISA sensitivity compares favorably with the EBL test. The lower detection limit of both tests is 25-50 pg PMT (54). Subcultures, producing only 0.1% of the PMT usually produced by toxigenic cultures *in vitro*, can be detected by this assay (56, 90).

The advantages of the PMT ELISA are many. It is as sensitive and specific as the EBL assay and is more reproducible. Single colonies from mixed cultures can be detected even when the sample is from heavily contaminated material. Technicians or bacteriologists expert in *P. multocida* identification are not needed (56). The test is independent of lab animal use, addressing animal welfare concerns. It is simple and rapid, with one technician able to perform the assay on large numbers of samples daily (169).

Anti-PMT antibody ELISAs measure titer in serum and body secretions, providing evidence of PMT exposure (55). Kimman et al. used an indirect double antibody sandwich ELISA for class specific anti-pseudorabies virus antibody detection in serum and respiratory secretions (92). Measurement of class specific anti-PMT antibody in serum and nasal secretions may be possible, giving an indication of PMT exposure. Although specific, the test cannot differentiate between seroconversion caused by infection or by toxoid immunization.

In addition to ELISA, an antibody based colony lift test has been developed by Magyar and Rimler (102). This test relies on antibody recognition of PMT absorbed onto a colony overlay supportive matrix.

<u>DNA probe</u> ToxA gene identification raises the possibility a DNA probe used as a diagnostic toxigenic *P. multocida* test, however test specificity has been questioned. Although some studies show 100% specificity of *in vitro* toxA probe hybridization to toxigenic strains (56, 116), areas of toxA gene homology in other bacterial species and some nontoxigenic strains have been found, causing false positive results (91).

Immunology of the Respiratory Mucosal Surface

Introduction

The idea of a local immunity system dates to the 1920s (14). Only during the last 20 years has the relevance of local immunity been applied to local *immunization* (14). Improved methods studying immunocyte origin and function have advanced the common mucosal immune system theory. New techniques enable specific immunoglobulin titration in secretions and serum.

Kimman et al. found the mucosal antibody responses in all mucosal secretions were highly similar after pseudorabies virus infection or vaccination (92). McDermott and Bienenstock identified mesenteric lymph node immune cells localizing in the gut, cervix, vagina, uterus, mammary gland and mesenteric lymph nodes. Lung origin cells were also found to localize in recipient lungs (111). These concepts are important to swine health, as they impact vaccination and disease prevention.

Mucosal defenses

Brandtzaeg defines the respiratory mucosal defenses based on functional divisions. The mucosal immune system works by immune exclusion, regulation and/or elimination (21).

Immune exclusion Immune exclusion uses nonspecific mechanisms, and specific responses such as immunoglobulins, to limit bacterial colonization and penetration at mucosal surfaces. Nonspecific examples include the mucus layer found on the upper respiratory tract ciliated epithelial cells. As mucus layer mechanical and hydrophilic properties attract and trap bacteria, ciliary action moves the mucus to the oropharynx for removal (16, 21).

Lysozyme and lactoferrin act within the mucus layer, killing bacteria or inhibiting growth. Lysozyme is bacteriocidal and bacteriostatic, accelerating lysis from antibody activated complement fixation (16, 21). Lactoferrin is a bacteriostatic iron binding protein, depriving pathogenic organisms of iron (21).

Cytotoxic T cells predominate in the respiratory epithelium. Possessing major histocompatibility class (MHC) I molecules, they are one of the first cells to contact foreign bodies and initiate an immune reaction (123).

Immunoglobulin type alpha Immunoglobulin type alpha (IgA) production and secretion is a specific mucosal immune system response to antigen exposure. IgA is not as highly specific as the systemic immunoglobulin type gamma (IgG). Wider specificity range is an advantage when dealing with organisms prone to antigenic drift (21).

IgA associated with a bacteria reduces its negative surface charge, increases its hydrophilicity, promotes hydrophilic mucus layer entrapment and prevents adherence and colonization (16, 21, 89). Mucosal surface IgA is of significant protective value against superficially replicating organisms (16).

IgA neutralizes certain exotoxins and respiratory viruses (21, 89). *In vitro* viral neutralization properties are weak. IgA might be more important *in vivo* in trapping toxin and viral particles within the mucus layer and enabling clearing, rather than preventing viral infection by neutralization (92).

Another IgA protective property is its effective antigen cross linking capabilities, promoting microbial agglutination and blocking soluble material and allergen absorption (21, 89). This is especially important in preventing immune mediated respiratory tract pathology. IgA fixation on bacterial surfaces prevents the potent complement activator, IgG, from binding and initiating complement component release, which can damage host tissues. IgA reduces hydrophobic interactions, decreasing the association of phagocytic sensitive bacteria with neutrophils and preventing continued pathogenic interleukin release (16). Increasing goblet cell mucus release, limiting bacterial multiplication by antibody dependent cytotoxicity and suppressing immune cell chemotaxis, are additional IgA properties helping safeguard against pathogenic mucosal immune reactions (21).

Respiratory mucous membrane IgA is primarily in dimer form. Two monomeric IgA molecules are bound together by a 12 kDa glycoprotein known as the "J chain" to form the dimer (157).

An 80 kDa glycoprotein called the secretory component (SC) is attached at the J chain-IgA junction. SC, homologous to other proteins in the immunoglobulin superfamily, is the ectoplasmic domain of the polymeric IgA receptor found on the basal side of mucosal glandular epithelium. It also functions as a stearic inhibitor of proteolytic enzymes found in the respiratory tract mucus layer (157).

Using immunohistochemistry, Brandtzaeg found human glandular epithelial cells contain membrane associated IgA and SC. Free SC was found within the cell Golgi apparatus while intracellular SC outside the Golgi region was coupled with dimeric IgA (20).

These and other findings, as detailed by Solari and Kraehenbul (157), support the following IgA secretion mechanism: SC is synthesized on the rough endoplasmic reticulum of the serous secretory epithelial cells. After transport to the Golgi apparatus, where it accumulates and undergoes a maturation process, free SC is moved to the basal cell membrane. IgA is produced by immunocytes next to the basal cell membrane and released in the dimeric form, associated with a J chain. Membrane associated SC acts as an epithelial J chain receptor causing specific affinity for dimeric IgA by the basal cell membrane. The SC-IgA complex is endocytosed, moving to and fusing with the apical plasma membrane. The SC receptor anchoring domain is cleared, releasing into the glandular lumen dimeric IgA still bound to the ectoplasmic SC receptor domain (157).

IgA immunocytes are particularly prevalent around the glandular cells located deep in the mucosa (16) with some specific differences between the distribution of IgA and IgG. IgA has been regularly located between these cells, whereas IgG has irregularly been identified in these same areas (20). Also, large IgG concentrations are found in interstitial fluid and at the epithelial basement membrane zones, with virtually no uptake by the glandular acinar cells seen as with IgA.

Immunoglobulin type gamma Because of its low molecular weight, IgG appears to diffuse as a transudate from serum into bronchial secretions (89). This occurs by passive diffusion through the epithelial intercellular tight junctions during normal physiologic conditions and, most particularly, during inflammation when these tight junctions become more permeable (16, 20, 21, 89).

IgG has a role in immune exclusion. If antigen neutralization or elimination is not successful, increasing mucosal permeability facilitates foreign material influx between cells. Breakdown of mucosal integrity may continue by complement mediated immunopathologic mechanisms (21). As this process occurs there is an increase in serum IgG exudation through capillary fenestrations, between mucosal cells and into the respiratory lumen, preventing further antigen invasion. (21)

The mucus layer carries both IgA and IgG. The IgG source is from either transudation, exudation in the presence of inflammation, or local synthesis by submucosal immunocytes (89). Mucosal IgA is either monomeric, from serum, or dimeric and actively transported across the respiratory epithelium. Due to the lack of differentiating features, it is impossible to accurately determine exact amounts of immunoglobulins derived from these sources (89).

Immune regulation Immune regulation is a process resulting from antigen presenting cells (APC) and lymphocyte T and B cell interactions. Self and non-self antigens are recognized and particular antibody classes (A, D, G or E) are produced and released (21).

T helper cells Upper respiratory tract APCs are responsible for holding or processing antigens in a manner needed for T helper (T_h) cell interaction. APCs include macrophages, dendritic cells (Langerhans cells) and certain epithelial cells (an example of which is present in human tonsillar tissue) (21). They present foreign antigens and HLA-like (Human Leukocyte Antigens - a key to immune system "self" recognition) native cell determinants to T_h cells, which in turn help stimulate B cell responses to the foreign antigens (21).

T_h cells with $CD4^+$ phenotype are the predominant T lymphocytes in the lamina propria (124). These cells recognize foreign antigen through the MHC class II molecule (123). Mucosal IgA induction is dependent on cognate help provided by these $CD4^+$ T cells (123, 139). The $CD4^+$ cells also release lymphokines coordinating the immune function, altering the normal respiratory physiology (123) and stimulating T suppressor cells, to inhibit other than the IgA producing B cell isotype (21).

B cells destined to produce secretory IgA must be continually resupplied to the respiratory submucosa, maintaining the secretory IgA supply necessary for extended local immune protection. The nasal mucosa has the cells necessary for antibody response, but it is not known whether the secretion immune response is the result of local nasal mucosal or oropharynx lymphoepithelial tissue production (45). McDermott and Bienenstock (111) and Berstein (16) showed the ability of immature B cells, with a potential for J chain production, to become stimulated in mediastinal lymph nodes, tonsillar and adenoid tissues and then migrate to glandular sites where they are subjected to terminal differentiation. The predominant immunoglobulin content of these cells is IgA. Brandtzaeg (21) also contends humerally circulating J chain positive B cell blasts are continuously seeded into the nasal stroma. Signals mediated by locally activated T cells, antigens, mitogens, or local hormones then induce B cell differentiation in the tissue. Most of these B cells end as IgA dimer producing immunocytes, with a few J chain positive IgG, IgD and IgM producing immunocytes also developing.

Interleukin Ramsey et al. think T_h cell produced interleukin 6 (IL-6) is one of the primary development signals for *in vivo* mucosal antibody response. By identifying a mouse genetic line incapable of producing IL-6, they found the capacity of the mutant mice to mount specific intestinal IgA antibody containing cell responses to be grossly deficient. Mutant IL-6$^-$ mice had substantially fewer IgA plasma cells in their intestines, mesenteric lymph nodes and lungs compared to IL-6$^+$

mice. Finally, the IgA response in the mutant mice was completely restored after intranasal infection with recombinant vaccinia virus, engineered to express IL-6 (139).

Immune elimination Immune elimination is the functional property of the mucosal immune system addressing foreign material neutralization and elimination after entering the body. IgG phagocytosis enhancement and antibody dependent cytotoxicity involving complement or killer cells assist this action.

In contrast to the superficial and glandular associated IgA producing B cells, the IgG immunocytes are present in relatively large numbers in the deeper lamina propria layer. During inflammatory rhinitis the number of these IgG producing cells increases. Associated IgG has a hydrophobic effect on the bacterial surface, promoting engulfment by phagocytic cells. Persistent IgG immune complexes increase capillary and epithelial permeability, allowing immunoglobulin exudation and entry of mucosal surface bystander antigen into the submucosa (16).

Summary of mucosal defense mechanisms In summary, the mucosal immune system functional components, immune exclusion, regulation and elimination, must be active and effective to ensure mucosal protection against infection. Two "lines of defense" are operable. The first includes nonspecific bacteriocidal or bacteriostatic properties of the respiratory mucus coat, lysozyme, lactoferrin and other factors. More specific response involves local production of J chain IgA next to SC bearing epithelium (21). These factors, and the inhibitory action by the indigenous bacterial flora, have a synergistic effect in preventing upper respiratory tract bacterial adherence and colonization (16).

The second line comes into play with the initiation of inflammation. Serum transferred antibodies and protective factors in the mucosal interstitial fluid act to eliminate submucosal and epithelial antigenic materials (21).

Secretory immunoglobulins

Type of immunoglobulins on mucosal surfaces Nasal secretion and serum immunoglobulin content in the newborn and growing pig has been investigated by Morgan and Bourne (114). Without addressing vaccination history and using nasal lavage to collect secretions, they reported class specific antibody ratios. IgG is, quantitatively, the major immunoglobulin in the nasal secretions of these pigs, agreeing with studies of human babies, lambs and calves (114). Using IgA:IgG ratios to follow relative immunoglobulin level, a transient IgA predominance in nasal secretions was seen during the first week of life. The most probable explanation is passively acquired IgA selectively being transported to the respiratory tract. The IgA:IgG ratio decreases to a low at five weeks (0.25:1). Predominance of porcine respiratory tract IgG during two to ten weeks of age is a reflection of low IgA content, not elevated IgG levels. About the fifth week, local IgA synthesis becomes active, increasing the IgA:IgG ratio until by 11 weeks of age the ratio is greater than 1:1. The major immunoglobulin in the adult pig upper respiratory tract secretions is IgA (113).

Using an ELISA to assay for antibodies in pig serum and bronchoalveolar fluid after infection with *Actinobacillus pleuropneumonia*, Loftager and Erisksen found mucosal IgA response rises before a systemic antibody response (99). During early stages of natural infection, IgA was present in bronchoalveolar fluid and saliva, when no serum antibodies were detectable. Later in infection, when antibodies were present in serum, very low but detectable IgA levels were found in the bronchoalveolar fluid and none in saliva (99). Balfour-Lynn et al. contend that, in the human, such an increase in mucosal IgA during the very early infection period is from plasma exudation rather than increased IgA production (13).

Kimman et al. measured mucosal and serum response to vaccination with pseudorabies virus (92). They found pseudorabies specific IgA in samples for variable periods, however, serum IgG response persisted. Intranasal vaccination led to low lung, nasal, tear and saliva IgA titers, but an IgA anamnestic response was seen following challenge 96 days post-vaccination.

<u>Collection of samples</u> Many studies examining antibody content in nasal secretions have used nasal lavage to collect samples (13, 48, 92, 99, 113, 114). Morgan and Bourne investigated the influence collection

method has on immunoglobulin levels in the pig nasal and tracheal secretions (113). Nasal lavage was done by collection of instilled fluids as they ran out of the nasal cavity while the pig was in a head down, dorsally recumbent position. Collection was also done by placing a tampon in the nasal passage and expressing the collected fluids. Comparison of these two methods showed the IgA:IgG ratio in the tampon samples to be significantly less than in nasal lavage samples. In spite of the absence of blood, epithelial damage caused by tampons resulted in leakage of IgG rich interstitial fluid into the secretion (113).

Bibliography

1. Aakai, T., A. Sawata, M. Tsuji, Y. Somejima and K. Kume. 1984. Purification of dermonecrotic toxin from a sonic extract of *Pasteurella multocida* SP-72 serotype D. Infection and Immunity. 46: 429-434.

2. Ackermann, M.R., D.A. Adams, L.L. Gerken, M.J. Beckman and R.B. Rimler. 1993. Purified *Pasteurella multocida* protein toxin reduces acid phosphatase-positive osteoclasts in the ventral nasal concha of gnotobiotic pigs. Calcif Tissue Int. **52** (6): 455-459.

3. Ackermann, M.R., M.F. Cheville and J.E. Gallagher. 1991. Colonization of the pharyngeal tonsil and respiratory tract of the gnotobiotic pig by a toxigenic strain of *Pasteurella multocida* type D. Vet Pathol. **28**: 267-274.

4. Ackermann, M.R., M.C. DeBey and K.B. Register. 1995. Reduced physial area and chondrocyte proliferation in *Pasteurella multocida* toxin treated rats. Vet Path. **32:** 674-682.

5. Ackermann, M.R., M.C. DeBey, K.B. Register, D.J. Larson and J.M. Kinyon. 1994. Tonsil and turbinate colonization by *Pasteurella multocida*. J Vet Diag Invest. **6:** 375-377.

6. Ackermann, M.R., R.B. Rimler, J.R. Thurston. 1991. Experimental model of atrophic rhinitis in gnotobiotic pigs. Infection and Immunity. **59**: 3626-3629.

7. Baalsrud, K.J. 1987. Vaccination against atrophic rhinitis: effect on clinical symptoms, growth rate and turbinate atrophy. Acta Vet Scand. **28**: 305-311.

8. Bäckström, L.R., T.A. Brim and M.T. Collins. 1988. Development of turbinate lesions and nasal colonization by *Bordetella bronchiseptica* and *Pasteurella multocida* during long-term exposure of healthy pigs to pigs affected by atrophic rhinitis. Can J Vet Res. **52**: 23-29.

9. Bäckström, L., H. Bremer, I. Dryendahl and H. Olsson. 1976. Atrophic rhinitis in pigs. The relationship between atrophic rhinitis, weight gain, age of the dam and genetic dispositions in a swine pedigree herd with a high incidence of the disease. Svensk Vet Tidn. **28**: 449-455.

10. Bäckström, L. and D.C. Hoefling. 1986. Transmission of atrophic rhinitis in swine under different environmental conditions. Agri-practice. **7**: 23-27.

11. Bäckström, L., D. Hoefling and A. Morkoc. 1982. Atrophic rhinitis in swine I: clinical signs, slaughter lesions, daily weight gain, disease transmission. Proceedings Int Pig Vet Soc Congr. Mexico City. p 102.

12. Bäckström, L., D.C. Hoefling, A. Morkoc and R. Cowart. 1985. Effect of atrophic rhinitis on growth rate in Illinois swine herds. J Am Vet Med Assoc. **187**: 712-715.

13. Balfour-Lynn, I.M., B. Volman, M. Silverman and A.D. Webster. 1993. Nasal IgA response in wheezy infants. Arch Dis Child. **68**: 472-476.

14. Bang, B.G. and F.B. Bang. 1977. Nasal mucociliary systems. pp 405-426. **Respiratory Defense Mechanisms**, part I. J.D. Brain, D.F. Proctor and L.M. Reid, eds. Marcel Dekker, Inc., New York.

15. Beckmann, G. and P. Schöss. 1988. Investigations on the occurrence of toxigenic *Pasteurella multocida* strains in tonsils and noses of piglets in atrophic rhinitis unsuspicious herds. Dtsch tierärztl Wschr. **95**: 283-285.

16. Berstein, J.M. 1992. Mucosal immunology of the upper respiratory tract. Respiration. **59 (suppl. 3)**: 3-13.

17. Bording, A and N.T. Foged. 1991. Characterization of the immunogenicity of formaldehyde detoxified *Pasteurella multocida* toxin. Vet Micro. **29**: 267-280.

18. Bording, A., K. Nymark and E. Smidt. 1994. Field trials with a new genetically engineered vaccine for protection against progressive atrophic rhinitis in pigs. Acta Vet Scand. **35** (2): 155-163.

19. Bowerstock, T.L., T. Hooper and R. Pottenger. 1992. Use of ELISA to detect toxigenic *Pasteurella multocida* in atrophic rhinitis in swine. J Vet Diag Invest. **4** (4): 419-422.

20. Brandtzaeg, P. 1974. Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epithelial cells. J. of Immunology. **112**: 1553-1559.

21. Brandtzaeg, P. 1984. Immune functions of human nasal mucosa and tonsils in health and disease. pp 28-95. **Immunology of the lung and upper respiratory tract**. J. Bienenstock, ed. McGraw-Hill Book Co., New York.

22. Briggs, R.E. and G.H. Frank. 1992. Increased elastase activity in nasal mucus associated with nasal colonization by P. Haemolytica in infectious bovine rhinotracheitis virus - induced calves. Am J Vet Res. **53**: 631-635.

23. Brown, W.R., L. Krook and W.G. Pond. 1966. Atrophic rhinitis in swine. Etiology, pathogenesis and prophylaxis. Cornell Vet. (Suppl 1). **56**: 1-107.

24. Buys, W.E., H.E. Smith, A.M. Kamps, E.M. Kamp, M.A. Smits. 1990. Sequence of the dermonecrotic toxin of *Pasteurella multocida* ssp multocida. Nucleic Acids Res. **18**: 2815.

25. Carter, G.R. 1955. Studies on *Pasteurella multocida*. I. A. hemagglutination test for the identification of serological types. Am J Vet Res. **16**: 481-484.

26. Chanter, N. 1990. Molecular aspects of the virulence of *Pasteurella multocida* . Can J Vet Res. **54:** 545-547.

27. Chanter, N. and J.M. Rutter. Atrophic rhinitis. pp 162-195. **Pasteurella and Pasteurellosis.** C. Adlam and J.M. Rutter, eds. Academic Press, London.

28. Chanter, N. and J.M. Rutter. 1989. Comparison of methods for the sampling and isolation of toxigenic *Pasteurella multocida* from the nasal cavity of pigs. Res in Vet Sci. **47**: 355-358.

29. Chanter, N. and J.M. Rutter. 1990. Colonization of *Pasteurella multocida* in atrophic rhinitis of pigs and immunity to the osteolytic toxin. Vet Micro. **25**: 253-265.

30. Chanter, N., J.M. Rutter and P.D. Luther. 1986. Rapid detection of toxigenic *Pasteurella multocida* by an agar overlay method. Vet Rec. **119**: 629-630.

31. Chanter, N., J.M. Rutter and A. Mackenzie. 1986. Partial purification of an osteolytic toxin from *Pasteurella multocida*. J Gen Micro. **132**: 1089-1097.

32. Cheville, N.F., R.B. Rimler and J.R. Thurston. 1988. A toxin from *Pasteurella multocida* type D causes acute hepatic necrosis in pigs. Vet Pathol. **25**: 518-520.

33. Chrisp, C.E. and N.T. Foged. 1991. Induction of pneumonia in rabbits by use of a purified protein toxin from *Pasteurella multocida*. Am J Vet Res. **52**: 56-61.

34. Chung, W., M. Collins and L. Bäckstrom. 1990. Adherence of *Bordetella bronchiseptica* and *Pasteurella multocida* to swine nasal ciliated epithelial cells *in vitro*. APMIS. **98**: 453-461.

35. Collins, M.T., L.R. Bäckström, T.A. Brim. 1989. Turbinate parameter ratio as an indicator of conchal atrophy for diagnosis of atrophic rhinitis in pigs. Am J Vet Res. **50**: 597-601.

36. Confer, A.W. 1993. Immunogens of Pasteurella. Vet Micro. **37** (3-4): 353-368.

37. Cowart, R., L. Bäckström and T. Brim. 1989. *Pasteurella multocida* and *Bordetella bronchiseptica* in atrophic rhinitis and pneumonia in swine. Can J Vet Res. **53**: 295-300.

38. Cowart, R., R.J. Lipsey and H. Hedrick. 1990. Measurement of conchal atrophy and pneumonia lesions and their association with growth rate in commingled feeder pigs. J Am Vet Med Assoc. **196**: 1262-1264.

39. deJong, M.F. 1983. Treatment and control of atrophic rhinitis in the Netherlands. in **Atrophic rhinitis of pigs**. K.B. Pedersen and N.C. Nielsen, eds. CEC Agriculture Eur. 8643 EN., EEC, Luxemborg. pp 165-176.

40. deJong, M.F. (Progressive) atrophic rhinitis. pp 414-435. **Diseases of Swine**, 7th Ed. A.D. Leman, B.E. Straw, W.L. Mengeling, S. Dallaire and D.J. Taylor, eds. Iowa State University Press, Ames, Iowa.

41. DeJong, M.F., M.L. Oie and G.J. Tentenburg. 1980. Atrophic rhinitis pathogenicity tests for *Pasteurella multocida* isolates. Proceedings 6th Int Pig Vet Soc Cong. Copenhagen. p 211.

42. DiGiacomo, R.F., B.J. Deep, W.E. Giddens, B.L. Bernard and M.M. Chengappa. 1989. Atrophic rhinitis in New Zealand white rabbits infected with *Pasteurella multocida* . Am J Vet Res. **50**: 1460-1465.

43. Dominick, M.A. and R.B. Rimler. 1986. Turbinate atrophy in gnotobiotic pigs intranasally inoculated with protein toxin isolated from type D *Pasteurella multocida* . Am J Vet Res. **47**: 1532-1536.

44. Done, J.T., D.H. Upcott, D.C. Frewin and C.N. Hebert. 1984. Atrophic rhinitis: snout morphometry for quantitative assessment of concal atrophy. Vet Rec. **114**: 33-35.

45. Durgal, F., M. Bilanger and M. Jacques. 1992. Enhanced adherence of *Pasteurella multocida* to porcine tracheal rings preinfected with *Bordetella bronchiseptica*. Can J Vet Res. **56**: 260-264.

46. Eamens, G., P.D. Kirkland, M.J. Turner, I.A. Gardner, M.P. White and C.L. Hornitzkyet. 1988. Identification of toxigenic *Pasteurella multocida* in atrophic rhinitis of pigs by in vitro characterization. Aust Vet J. **65**: 120-123.

47. Eliás B. 1986. Epidemiological studies on atrophic rhinitis in swine IX, investigations on the introduction of the disease. J Am Vet Med. **33**: 60-67.

48. Eliás, B., M. Kruger, P. Gergely, R. Voets and P. Rafai. 1993. Interaction between immunity to *Bordetella bronchiseptica* and infection of pig herds by *Bordetella bronchiseptica* and *Pasteurella multocida*. J Vet Med Sci. **55**: 617-622.

49. Elling, F. and K.B. Pedersen. 1985. The pathogenesis of persistent turbinate atrophy induced by toxigenic *Pasteurella multocida* in pigs. Vet Pathol. **22**: 469-474.

50. Elling, F. and K.B. Pedersen. 1983. Atrophic rhinitis in pigs induced by a dermonecrotic type A strain of *Pasteurella multocida*. in **Atrophic rhinitis of pigs**. K.B. Pedersen and N.C. Nielsen, eds. CEC Agriculture Eur. 8643 EN., EEC, Luxemborg. pp 123-135.

51. Elling, F., K.B. Pedersen, P. Høgh and N.T. Foged. 1988. Characterization of the dermal lesions induced by a purified protein from toxigenic *Pasteurella multocida*. APMIS. **96**: 50-55.

52. Fantino, M. and L. Wieteska. 1993. Evidence for a direct central anorectic effect of tumor necrosis factor alpha in the rat. Physiol and Behav. **53**: 477-483.

53. Fenwick, B.W. 1990. Virulence attributes of the lipopolysaccarides of the HAP-group organisms. Can J Vet Res. **54**: S28-S32.

54. Foged, N.T. 1988. Quantification and purification of the *Pasteurella multocida* toxin by using monoclonal antibodies. Infection and Immunity. **56**: 1901-1906.

55. Foged, N.T. 1991. Detection of stable epitopes on formaldehyde - detoxified *Pasteurella multocida* toxin by monoclonal antibodies. Vaccine. **9**: 817-823.

56. Foged, N.T. 1992. *Pasteurella multocida* toxin: the characterization of the toxin and its significance in the diagnosis and prevention of progressive atrophic rhinitis in pigs. AMPIS Suppl 25. **100**: 5-56.

57. Foged, N.T., J.P. Nielsen and K. Barfod. 1990. The use of ELISA determination of *Pasteurella multocida* toxin antibodies in the control of progressive atrophic rhinitis. Proceedings 11th Int Pig Vet Soc Congr, Lausanne. p 49.

58. Foged, N.T., J.P. Nielsen, K.B. Pedersen. 1988. Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by enzyme-linked immunosorbant assay. J of Clin Micro. **26**: 1419-1420.

59. Foged, N.T., K.B. Pedersen and F. Elling. 1987. Characterization and biological effects of the *Pasteurella multocida* toxin. FEMS Micro Letters. **43**: 45-51.

60. Frandsen, P.L., N.T. Foged, S.K. Petersen and A. Bording. Characterization of toxin from different strains of *Pasteurella multocida* serotype A and D. J Am Vet Med Assoc. **38**: 345-352.

61. Frymus, T., E. Müller, B. Franz, K. Petzoldt. 1989. Protection by toxoid-induced antibody of gnotobiotic piglets challenged with the dermonecrotic toxin of *Pasteurella multocida* . J Am Vet Med Assoc. **36**: 674-680.

62. Frymus, T., E. Müller and K. Petzoldt. 1989. Antigenic relationship between the dermonecrotic toxins produced by *Pasteurella multocida* type D and type A. J Am Vet Med. **36**: 199-202.

63. Gardner, I., G.J. Eamens, M.J. Turner and C.L. Hornitzky. Toxigenic type D *Pasteurella multocida* in New South Wales pig herds - prevalence and factors associated with infection. Aust Vet J. **66**: 318-321.

64. Gardner, I. and D. Hird. 1990. Host determinants of pneumonia in slaughter weight swine. Am J Vet Res. **51**: 1306-1311.

65. Gardner, I.A., R. Kasten, G.J. Eamens, K.P. Snipes and R.J. Anderson. 1994. Molecular fingerprinting of *Pasteurella multocida* associated with progressive atrophic rhinitis in swine herds. J Vet Diag Invest. **6** (4): 442-447.

66. Geiger, G. 1992. Elimination of toxigenic *Pasteurella* utilizing Isowean[SM] three site production. Proceedings American Association of Swine Practitioners, Nashville. pp 45-47.

67. Ghoshal, N., Y. Niyo and A. Trenkle. 1991. Growth hormone concentrations in plasma of healthy pigs and pigs with atrophic rhinitis. Am J Vet Res. **52**: 1684-1687.

68. Gois, M., D. Farrington, H. Barnes and R. Ross. 1983. Long acting oxytetracycline for control of induced *Pasteurella multocida* rhinitis in swine. J Am Vet Med Assoc. **183**: 445-447.

69. Goodwin, R.F.W. 1988. Monitoring for atrophic rhinitis: The problem of higher snout scores. Vet Rec. **123**: 566-568.

70. Goodwin, R.F.W., N. Chanter, J.M. Rutter. 1990. Detection and distribution of toxigenic *Pasteurella multocida* in pig herds with different degrees of atrophic rhinitis. Vet Rec. **126**: 452-456.

71. Goodwin, R.F.W. and P. Whittlestone. 1983. Monitoring for atrophic rhinitis: five years experience with a pilot control scheme. Vet Rec. **113**: 411-412.

72. Grund, S., P. Schoss and B. Thiesen. 1990. *Pasteurella multocida* (type D and A) and atrophic rhinitis of swine-hemagglutination, fimbrial and adhesion in vitro in the nasal mucosa of swine fetuses. Berl-Munch-Tierarztl-Wochenschr. **103**: 365-371.

73. Gutman, L.T. and H.P. Willet. 1992. *Pasteurella* and miscellaneous Gram-negative bacilli. pp 600-608. **Zinsser Microbiology**, 20th Ed. W.K. Joklik, H.P. Willett, D.B. Amos and C.M. Wilfert, eds. Appleton and Lange, Norwalk, CT.

74. Harel, J., S. Côtés and M. Jacques. 1990. Restriction endonuclease analysis of porcine *Pasteurella multocida* isolates in Quebec. Can J Vet Res. **54**: 422-426.

75. Harris, D.L., R.F. Ross and W.P. Switzer. 1969. Incidence of certain microorganisms in nasal cavities of swine in Iowa. Am J Vet Res. **30**: 1621-1624.

76. Harris, D.L., W.P. Switzer. 1968. Turbinate atrophy in young pigs exposed to *Bordetella bronchiseptica*, *Pasteurella multocida* and combined inoculum. Am J Vet Res. **29**: 777-785.

77. Hoffman, L.J. 1989. Recovery of toxigenic *Pasteurella multocida* and *Bordetella bronchiseptica* from Iowa swine herds affected with atrophic rhinitis. **Iowa State University 1989 Swine Research Report**. ASL-R660, paper ASL-R659. p 66-67.

78. Hoffman, L.J. 1990. Recoverability of *Pasteurella multocida* from nasal cavities of challenged pigs. **Iowa State University 1990 Swine Research Report**. ASL-R773, paper ASL-R773. p 63-64.

79. Hoie, S., K. Falk and B. Lium. 1991. An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. IV. Bacteriological findings in chronic pneumonic lesions. Acta Vet. Scand. **32**: 395-402.

80. Hu, S.P., L.J. Felice, V. Sivanandan and S.K. Maheswaran. 1986. Sidophore production by *Pasteurella multocida* . Infection and Immunity. **54**: 804-810.

81. iDali, C., N.T. Foged, P.L. Frandsen, M.H. Nielsen and F. Elling. 1991. Ultrastructural localization of the *Pasteurella multocida* toxin in a toxin producing strain. J of Gen Micro. **137**: 1067-1071.

82. Ikeda, J. and D. Hirsh. 1990. Possession of identical nonconjunctive plasmids by different isolates of *Pasteurella multocida* does not imply clonality. Vet Micro. **22**: 79-87.

83. Il'ina, Z.M. and I. Zasukhin. 1975. Role of *Pasteurella* toxins in the pathogenesis of infections atrophic rhinitis. Vet Inst Omak. **25**: 76-86.

84. Inzana, T.J. 1990. Capsules and virulence in the HAP-group of bacteria. Can J Vet Res. **54**: S22-S27.

85. Iowa data, National Animal Health Monitoring System. 1988-1989. USDA:APHIS:VS, Fort Collins, CO.

86. Jacques , M., N. Parent and B. Foiry. 1988. Adherence of Bordetella bronchiseptica and *Pasteurella multocida* to porcine nasal and tracheal epithelial cells. Can J Vet Res. **52**: 283-285.

87. Jolie, R., P. deRoose and N. Tryttens. 1990. Diagnosis of atrophic rhinitis by computerized tomography: A preliminary report. Vet Rec. **126**: 591-594.

88. Kabay, M.J., A.R. Mercy, J.M. Lloyd and G.M. Robertson. 1992. Vaccine efficacy for reducing turbinate atrophy and improving growth rate in piggeries with endemic atrophic rhinitis. Aust Vet J. **69**: 101-103.

89. Kaltreider, H.B. 1984. Local Immunity. p 191-215. **Immunology of the lung and upper respiratory tract**. J. Bienenstock,ed. McGraw-Hill Book Co., New York.

90. Kamp, E.M., E.A. terLoak and M.F. deJong. 1990. Atypical pasteurella strains producing toxin similar to the dermonecrotic toxin of *Pasteurella multocida* subspecies multocida. Vet Rec. **126**: 434-437.

91. Kamps, A.M.I.E., W.E.C.M. Buys, E.M. Kamp and M.A. Smits. 1990. Specificity of DNA probes for the detection of toxigenic *Pasteurella multocida* subsp. multocida strains. J Clin Micro. **28**: 1858-1861.

92. Kimman, T.G., R.A. Brouwers, J.J. Daus, J.T. vanOirschot and D. vanZaane. 1992. Measurement of isotype-specific antibody responses to Aujezky's disease virus in sera and mucosal secretions in pigs. Vet Immun and Immunopath. **31**: 95-113.

93. Kobisch, M. and A. Pennings. 1989. An evaluation in pigs of Nobi-Vac AR and an experimental atrophic rhinitis containing *Pasteurella multocida* DNT-toxoid and *Bordetella bronchiseptica*. Vet Rec. **124**: 57-61.

94. Kume, K. and T. Nakai. 1985. Dissociation of *Pasteurella multocida* dermonecrotic toxin into 3 polypeptide fragments. Japan J Vet Sci. **47**: 829-833.

95. Langhans, W. 1992. Acute phase response and inappetence. Ubersichten-zur-Tierernahrung. **20**: 39-64.

96. Lax, A.J. and N. Chanter. 1990. Cloning of the toxing gene from *Pasteurella multocida* and its role in atrophic rhinitis. J of Gen Micro. **136** (1): 81-87.

97. Lebrun, A., M. Caya and M. Jacques. 1992. Effects of sub-MICs of antibiotics on cell surface characteristics and virulence of *Pasteurella multocida*. Antimicro Agents Chemother. **36** (10): 2093-2098.

98. Letellier, A., D. Dubreuil, G. Roy, J.M. Fairbrother and M. Jacques. 1991. Determination of affinity of *Pasteurella multocida* isolates for porcine respiratory tract mucus, and partial characterization of receptors. Am J Vet Res. **52**: 34-39.

99. Loftager, M.-K. and L. Eriksen. 1993. Antibodies against *Actinobacillus pleuropneumoniae* serotype 2 in mucosal secretions and sera of infected pigs as demonstrated by an enzyme-linked immunosorbant assay. Res in Vet Sci. **54**: 57-62.

100. Love, R.J., M.R. Wilson and G. Tasler. 1985. Porcine atrophic rhinitis. Aust Vet J. **62**: 377-378.

101. Lugtenberg, B., R. vanBoxtel and M.F. deJong. 1984. Atrophic rhinitis in swine: Correlation of *Pasteurella multocida* pathogenicity with membrane protein and lipopolysaccharide patterns. Infection and Immunity. **46**: 48-54.

102. Magyar, T. and R.B. Rimler. 1991. Detection of toxin-producing *Pasteurella multocida*. J Clin Micro. **29**: 1328-1332.

103. Mannheim, W. 1984. Family: Pasteuellaceae. pp 550-558. **Bergey's Manual of Systematic Bacteriology, Vol 1**. N.R. Krieg and J.G. Holt, eds. Williams and Wilkins, Baltimore, MD.

104. Martineau, G.P., M. Denicourt, R. Charette, J. Lambert, A. Desilets, R. Sauvageau and G. Cousineau. 1989. Retrospective study on atrophic rhinitis and enzootic pneumonia from central testing station in Quebec, Part 3: effects on performance. Med Vet Quebec. **19**: 9-12.

105. Martineau-Doizé, B. G. Dumas, R. Larochelle, J.C. Frantz and G.P Martineau. 1991. Atrophic rhinitis caused by *Pasteurella multocida* type D: morphometric analysis. Can J Vet Res. **55**: 224-228.

106. Martineau-Doizé, B., J. Frantz and G. Martineau. 1990. Effects of purified *Pasteurella multocida* dermonecrotoxin on cartilage and bone of the nasal ventral conchae of the piglet. Anat Rec. **228**: 237-246.

107. Martineau-Doizé, B., J.C. Frantz and G.P. Martineau. 1990. Cartilage and bone lesions: An explanation of the severity of conchal atrophy induced by *Pasteurella multocida* , type D dermonecrotoxin. Proceedings 11th Int Pig Vet Soc Congr. Lausanne. p 79.

108. Martineau-Doizé, B., G. Martineau and A. Dhem. 1982. Study of the growth of the ventral nasal concha of the piglet by fluorescence microscopy and microradiography. Zbl Vet Med Comp Anat Histol Embryol. **11**: 193-204.

109. Martineau-Doizé. B., J. Menard, C. Girard, J.C. Frantz and G.P. Martineau. 1991. Effects of purified *Pasteurella multocida* dermonecrotic toxin on the nasal ventral turbinates of fattening pigs: histological observations. Can J Vet Res. **55**: 377-379.

110. Matschullat, G., E. Muller, J. Mumme and D. Ikes. 1994. Detection of toxin-producing strains of *Pasteurella multocida* in nasal and tonsil swabs, a possibility for the control of atrophic rhinitis in swine breeding herds? Parallel comparison with other research methods. DTW Dtsch Tierarztl Wochenschr. **101** (1): 27-30.

111. McDermott, M.R. and J. Bienenstock. 1979. Evidence for a common mucosal immunologic system. I. migration of B immunoblasts into intestinal, respiratory and genital tissues. J of Immunology. **122**: 1982-1898.

112. Mendoza, S., A. Ciprian, J. Camacho and J.A. Montaraz. 1993. Comparison of three biological assays for the detection of toxigenic properties of *Bordetella bronchiseptica* associated with atrophic rhinitis. Vet Micro. **36** (3-4): 215-219.

113. Morgan, K.L. and F.J. Bourne. 1980. Immunoglobulin levels in porcine nasal and tracheal secretions - the influence of the method of collection. J of Immun Meth. **37**: 165-173.

114. Morgan, K.L. and F.J. Bourne. 1981. Immunoglobulin content of the respiratory tract secretions of piglets from birth to 10 weeks old. Res in Vet Sci. **31**: 40-42.

115. Muller, R.D. and P.B. Abbott. 1986. Estimating the cost of respiratory disease in hogs. Anim Health Nutr. **41**: 30-35.

116. Nagai, S., S. Someno and T. Yagihashi. 1994. Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by PCR. J Clin Micro. **32** (4): 1004-1010.

117. Nakai, T., K. Kume and H. Yamada. 1986. Purification of dermonecrotic toxin from a sonic extract of *Pasteurella multocida* or *Bordetella bronchiseptica*. Proceedings 9th Int Pig Vet Soc Congr. Barcelona. p 222.

118. Nakai, T., K. Kume, H. Yoshikawa, T. Oyamada and T. Yoshikawa. 1988. Adherence of *Pasteurella multocida* or *Bordetella bronchiseptica* to swine nasal epithelial cell in vitro. Infection and Immunity. **56**: 234-240.

119. Nicolet, J. 1990. Overview of the virulence attributes of the HAP-group of bacteria. Can J Vet Res. **54**: S12-S15.

120. Nielsen, J.P., P. Korkman and S. Rosendal. 1992. Infection with toxigenic *Pasteurella multocida* and growth rate in a cohort of pigs. Proceedings Int Pig Vet Soc Congr. The Hague, Netherlands. p 32.

121. Nielsen, J.P., N.T. Foged, V. Sorense, K. Barfod, A. Bording and S.K. Petersen. 1991. Vaccination against progressive atrophic rhinitis with a recombinant *Pasteurella multocida* toxin derivative. Can J Vet Res. **55**: 128-138.

122. Nielsen, R., T. Plambeck and N.T. Foged. 1991. Blocking enzyme-linked immunosorbant assay for detection of antibodies to *Actinobacillus pleuropneumoniae* serotype 2. J Clin Micro. **34**: 794-797.

123. Okuda, M. and R. Powankar. 1992. Flow cytometric analysis of intraepithelial lymphocytes in the human nasal mucosa. Allergy. **47**: 255-259.

124. Pawankar, R. and M. Okuda. 1993. A comparative study of the characteristics of intraepithelial and lamina propria lymphocytes of the human nasal mucosa. Allergy. **48**: 99-105.

125. Pedersen, K.B. and K. Barfod. 1981. The aetiological significance of *Bordetella bronchiseptica* and *Pasteurella multocida* in atrophic rhinitis in swine. Nord Vet. **33**: 513-522.

126. Pedersen, K.B. and K. Barfod. 1982. Effect on the incidence of atrophic rhinitis of vaccination of sows with a vaccine containing *Pasteurella multocida* toxin. Nord Vet. **34**: 293-302.

127. Pedersen, K.B. and K. Barfod. 1984. Influence of vaccination of sows with *Bordetella-Pasteurella* vaccines on the occurrence of atrophic rhinitis among their offspring after experimental infection with *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida*. Nord Vet Med. **36**: 337-345.

128. Pedersen, K.B. and F. Elling. 1984. The pathogenesis of atrophic rhinitis in pigs induced by toxigenic *Pasteurella multocida*. J Comp Path. **94**: 203-214.

129. Pedersen, K.B., J.P. Nielsen, N.T. Foged, F. Elling, N.C. Nielsen and P. Willeberg. 1988. Atrophic rhinitis in pigs: proposal for a revised definition. Vet Rec Letters. **122**: 190-191.

130. Pejsak, Z., A. Hogg, B. Wasiska and K. Fordman. 1990. Comparison of six different regimens for the control of atrophic rhinitis in swine. J Am Vet Med Assoc. **37**: 593-598.

131. Petersen, S.K. and N.T. Foged. 1989. Cloning and expression of the *Pasteurella multocida* toxin gene tox A, in Escherichia coli. Infection and Immunity. **57**: 3907-3913.

132. Petersen, S.K., N.T. Foged, A. Bording, J.P. Nielsen, H.K. Riemann and P.L. Frandsen. 1991. Recombinant derivatives of *Pasteurella multocida* toxin: candidates for a vaccine against progressive atrophic rhinitis. Infection and Immunity. **59**: 1387-1393.

133. Pettit, R.K., R.B. Rimler and M.R. Ackermann. 1993. Protection of *Pasteurella multocida* dermonecrotic toxin-challenged rats by toxoid-induced antibody. Vet Micro. **34** (2): 167-173.

134. Philips, C.E. 1946. Infectious rhinitis in swine (bull nose). Can J Comp Med Vet Sci. **10**: 33.

135. Pijoan C. 1986. Effect of *Pasteurella multocida* and *Haemophilus pleuropneumonia* toxins on swine alveolar macrophages. Vet Immun and Immunopath. **13**: 141-149.

136. Pijoan, C., A. Lastra, C. Ramirez and A.D. Lemen. 1984. Isolation of toxigenic strains of *Pasteurella multocida* from lungs of pneumonic swine. J Am Vet Med Assoc. **185**: 522-523.

137. Pijoan, C. and F. Trigo. 1990. Bacterial adhesion to mucosal surfaces with special reference to *Pasteurella multocida* isolates from atrophic rhinitis. Can J Vet Res. **54 (Suppl)**: 516-21.

138. Plata-Salaman, C.R. 1991. Immunoregulators in the nervous system. Neurosci and Biobehav Review. **15**: 185-215.

139. Ramsey, A.J., A.J. Husband, I.A. Ramshaw, S. Bao, K.I. Matthaei, G. Koehler and M. Kopf. 1994. The role of interleukin-6 in mucosal IgA antibody responses *in vivo.* Science. **264**: 561-563.

140. Register, K.B., M.R. Ackermann and M.E. Kehrli, Jr. 1994. Non-opsonic attachment of *Bordetella bronchiseptica* mediated by CD11/CD18 and cell surface carbohydrates. Micro Pathogen. **17** (6): 375-385.

141. Rimler, R.B. and K.R. Rhoades. 1992. *Pasteurella multocida.* p 37-73. **Pasteurella and Pasteurellosis,** C. Adlam and J.M. Rutter, eds. Academic Press, London.

142. Robertson, J.F., D. Wilson and W.J. Smith. 1990. Atrophic rhinitis: the influence of the aerial environment. Anim Prod. **50**: 173-182.

143. Rosendal, S., P.L. Frandsen, J.P. Nielsen and R. Gallily. 1995. *Pasteurella multocida* toxin induces IL-6, but not IL-1 alpha or TNF alpha in fibroblasts. Can J Vet Res. **59** (2): 154-156.

144. Rutter, J.M. 1983. Virulence of *Pasteurella multocida* in atrophic rhinitis of gnotobiotic pigs infected with *Bordetella bronchiseptica.* Res in Vet Sci. **34**: 287-295.

145. Rutter, J.M. 1985. Atrophic rhinitis in swine. Adv Vet Sci Comp Med. **29**: 239-279.

146. Rutter, J.M. and P.D. Luther. 1983. Identification of toxigenic *Pasteurella multocida*. Vet Rec Letters. **113**: 304.

147. Rutter, J.M. and P.D. Luther. 1984. Cell culture assay for toxigenic *Pasteurella multocida* from atrophic rhinitis of pigs. Vet Rec. **114**: 393-396.

148. Rutter, J.M. and A. Mackenzie. 1984. Pathogenesis of atrophic rhinitis in pigs: a new perspective. Vet Rec. **114**: 89-90.

149. Rutter, J.M. and H. Rojas. 1982. Atrophic rhinitis in gnotobiotic piglets: Differences in the pathogenicity of *Pasteurella multocida* in combined infections with *Bordetella bronchiseptica*. Vet Rec. **110**: 531-535.

150. Sakano, T., A. Taneda, M. Okada, M. Ono, Y. Hayashi and S. Sato. 1992. Toxigenic type A *Pasteurella multocida* as a causative agent of nasal turbinate atrophy in swine. J Vet Med Sci. **54**: 403-407.

151. Scharmann, W.R., R. Drzeniek and H. Blobel. 1970. Neuraminidase of *Pasteurella multocida* . Infection and Immunity. **1**: 319-320.

152. Scheidt, A.B., V.B. Mayrose, M.A. Hill, L.K. Clark, T.R. Cline, K.E. Knox, L.J. Runnels, S. Frantz and M.E. Einstein. 1990. Relationship of pneumonia on atrophic rhinitis measured at slaughter with growth performance in swine. J Am Vet Med Assoc. **196**: 881-884.

153. Scheidt, A.B., V.B. Mayrose, M.A. Hill, L.K. Clark, M.E. Einstein, S.F. Frantz, L.J. Runnels and K.E. Knox. 1992. Relationship to growth performance of pneumonia and atrophic rhinitis lesions detected in pigs at slaughter among 4 seasons. J Am Vet Med Assoc. **200**: 1492-1496.

154. Schwartz W.L. and J.D. Williams. Oct, 1975. Atrophic rhinitis: significance of nasal measurements in specific-pathogen-free (SPF) swine herds. Vet Med/Sm Ani Clinician. pp 1213-1216.

155. Smith, R.D. 1991. *Sampling Methods*. pp 22-48. **Veterinary Clinical Epidemiology**. Butterworth-Heinemann. Boston, Mass.

156. Söderlind, O., M. Norquist and B. Thofoclin. Analysis of Bordetella and Pasteurella in the nostril flora by use of representative tampon samples as complement to sow-piglet herd control. In Proceedings of the 6th International Congress on Animal Hygiene, Skara, Sweden, June 1988, edited by I. Ekesbo, pp 250-253. Skara: Swedish University of Ag Sci, 1988.

157. Solari, R. and J.P. Kraehenlenl. 1990. Receptor-mediated transepithelial transport of polymeric immunoglobulins. **Molecular Biology of the Mammary Gland. Publisher.** pp 269-297.

158. Straw, B., E. Burgi, H. Hilley, A.D. Leman. 1983. Pneumonia and atrophic rhinitis in pigs from a test station. J Am Vet Med Assoc. **182**: 607-611.

159. Straw, B., A. Leman and R. Robinson. 1984. Pneumonia and atrophic rhinitis in pigs from a test station - a follow-up study. J Am Vet Med Assoc. **185**: 1544-1546.

160. Switzer, W.B. 1963. Disease of baby pigs. Dtsch Tierärztl Wschr. **70**: 216-219.

161. Thomson, C.M.A., N. Chanter and C.M. Wathes. 1992. Survival of toxigenic *Pasteurella multocida* in aerosols and aqueous liquids. Applied and Environ Micro. **58**: 932-936.

162. Trigo, E. and C. Pijoan. 1988. Presence of pili in *Pasteurella multocida* strains associated with atrophic rhinitis. Vet Rec. **122**: 19.

163. Turk J., M. Turk, W. Fales, L. Kintner, S. Nelson, D. Shaw, T. Brown and L. Morehouse. 1989. Summary of porcine respiratory disease diagnoses at the Missouri veterinary medical diagnostic laboratory for the years 1984 and 1986. J Vet Diagn Invest. **1**: 182-184.

164. Uhlenhopp, E.H. 1994. personal communication. Iowa State University College of Veterinary Medicine. Ames, Iowa.

165. van Diemen, P.M., M.F. de Jong, G. de Vries-Reilingh, P. van der Hel and J.W. Schrama. 1994. Intranasal administration of *Pasteurella multocida* toxin in a challenge-exposure model used to induce subclinical signs of atrophic rhinitis in pigs. Am J Vet Res. **55** (1): 49-54.

166. van Diemen, P.M., G. de Vries-Reilingh and H.K. Parmentier. 1994. Immune responses of piglets to *Pasteurella multocida* toxin and toxoid. Vet Immun Immunopath. **41** (3-4): 307-321.

167. van Diemen, P.M., A.M. Henken, J.W. Schrama, H.A. Brandsma and M.W. Verstegen. 1995. Effects of atrophic rhinitis induced by *Pasteurella multocida* toxin on heat production and activity of pigs kept under different climatic conditions. J Ani Sci. **76** (6): 1658-1665.

168. Varga, J., K. Magyar, L. Fodor and A. Romvary. 1991. Prevention and treatment of atrophic rhinitis in pigs with getroxel, chlorquinaldol and oxytetracycline. Acta Vet Hung. **39**: 127-135.

169. Vena, M.M., B. Blanchard, D. Thomas and M. Kobisch. 1991. Adherence of *Pasteurella multocida* isolated from pigs and relationship with capsular type and dermonecrotic toxin production. Ann Rich Vet. **22**: 211-218.

170. Voets, M.T., C.H. Klaassen, P. Charlier, A. Wislman and J. Descamps. 1992. Evaluation of an atrophic rhinitis vaccine under controlled conditions. Vet Rec. **130**: 549-553.

171. Ward, P.N., T.E. Higgins, A.C. Murphy, P.B. Mullan, E. Rozengurt, and A.J. Lax. 1994. Mutation of a putative ADP-ribosylation motif in the *Pasteurella multocida* toxin does not affect mitogenic activity. FEBS - Lett. **342** (1): 81-84.

172. Williams, P., M. Hall and R. Rimler. 1990. Host response to *Pasteurella multocida* turbinate atrophy toxin in swine. Can J Vet Res. **54**: 157-163.

173. Wilson, M.A., R.B. Rimler and L.J. Hoffman. 1992. Comparison of DNA fingerprints and somatic serotypes of serogroup B and E *Pasteurella multocida* isolates. J of Clin Micro. **30**: 1518-1524.

174. Wilson, M.R., R. Takov, R.M. Friendship, S.W. Martin, I. McMillan, R.R. Hacker and S. Swaminathan. 1986. Prevalence of respiratory diseases and their association with growth rate and space in randomly selected swine herds. Can J Vet Res. **50**: 209-216.

175. Yoshikawa, T. and T. Harada. 1981. Histopathological studies on pigs with atrophic rhinitis showing retarded growth. Jpn J Vet Sci. **43**: 221-231.

176. Zhao, G., L. Galina, W. Hanyanun and C. Pijoan. 1993. Human tonsillitis associated with porcine *Pasteurella multocida* infection. The Lancet. **342**: 491.

177. Zhao, G., C. Pijoan and M.P. Murtaugh. 1992. Epidemiology of *Pasteurella multocida* in a farrow-to-finish swine herd. Can J Vet Res. **57**: 136-138.

178. Zhao, G., C. Pijoan, M.P. Murtaugh and T.W. Molitar. 1992. Use of restriction endonuclease analysis and ribotyping to study epidemiology of *Pasteurella multocida* in closed swine herds. Infection and Immunity. **60**: 1401-1405.

III. SYSTEMIC AND LOCAL CLASS SPECIFIC ANTI-*PASTEURELLA MULTOCIDA* TOXIN ANTIBODY RESPONSE FOLLOWING TOXOID VACCINATION IN GILTS FED BETA-HYDROXY BETA-METHYL BUTYRATE

A paper prepared for the journal Infection and Immunity

Paul L. Sundberg, Eldon K. Uhlenhopp,
Steven L. Nissen, Lorraine J. Hoffman,
and John C. Fuller

Abstract

Thirty-six Landrace by Large White cross gilts were randomly assigned to one of three consecutive time blocks, 12 gilts per block. Within each block, gilts were randomly assigned to one of three beta-hydroxy beta-methyl butyrate (HMB) treatments (0 gm, 2 gm or 10 gm) top-dressed on the feed once daily, beginning 38 days before the expected block farrowing date and continuing through 21 days post-farrowing. They were housed in groups of three in separate isolation rooms before farrowing, and farrowed in a common room with 12 individual farrowing crates. Within each block each gilt was vaccinated with a commercially available killed pseudorabies (PRV) vaccine and a tetanus toxoid at separate sites. To test the effect of HMB treatment on immunoglobulin class specific humoral and mucosal antibody titers, gilts in each HMB treatment group were randomly assigned to be either *P. multocida* vaccinated or non-vaccinated.

Serum samples were collected on days –38 (before beginning HMB treatment), –28, –14, and –7 prefarrowing, and on days 1, 7, 14, and 21

postfarrowing. Collection of nasal samples was successful on days 38 prefarrowing, and days 1, 7, 14 and 21, postfarrowing. PRV and tetanus antibody titers were obtained on the sera using an ELISA test. A modification of a commercially available ELISA designed for detection of *P. multocida* toxin was used to find anti-PMT class specific antibody titer in the sera and nasal secretions.

Serum concentrations of HMB were in proportion to the level of HMB fed ($p<0.01$). The anti-tetanus and anti-PRV titers in those pigs fed 2 grams of HMB per day tended to be higher than that of the other two HMB treatment groups.

Treatment by feeding HMB appeared to have a suppressing effect on sera anti-PMT IgA. Sera anti-PMT IgG was not affected by HMB treatment.

Nasal anti-PMT IgA levels were also variable, with no discernable differences among the HMB treatment groups. Anti-PMT IgG levels varied but tended to be greater in pigs receiving no HMB on day 1, with no discernable differences on the following days.

There was interaction effect between HMB treatments and vaccination on the levels of sera IgA, day 21, and nasal IgA, day 14, with those gilts not being vaccinated tending to have the higher titers.

In this study, HMB vaccination with the particular *P. multocida* bacterin-toxoid used did not effect the level of anti-PMT antibodies in the nasal secretions or the sera. Oral HMB treatment with 2 grams per day tended to increase response to tetanus toxoid and PRV vaccinations.

Introduction

Release of *Pasteurella multocida* toxin (PMT) follows natural and experimental infection with the toxigenic strain of *P. multocida* in the upper respiratory tract of pigs (9). This toxin can cause progressive atrophic rhinitis (PAR) in pigs by affecting the bony nasal turbinate osteoblasts and osteoclasts. Intramuscular and intraperitoneal injection of purified toxin has been shown to cause turbinate atrophy typical of PAR, suggesting the toxin can travel hematogenously (5, 16, 17, 25).

P. multocida bacterins, alone and in combination with *Bordetella bronchiseptica*, have been used with varying degrees of success to prevent PAR (2). Including a toxoid fraction to these bacterins has increased the effectiveness of PAR vaccination programs (14, 19, 23).

PMT is antigenic and causes humoral antitoxin production when given intramuscularly to pigs (10). Anti-PMT antitoxin, given to pigs intraperitoneally, is protective against turbinate atrophy after intramuscular challenge with PMT and after experimental infection with toxigenic type D *P. multocida* (7, 10). This suggests circulating antibody may protect piglets against PMT that is produced in body sites such as the lungs or tonsils and reaches the nasal turbinates hematogenously.

While the primary immunoglobulin in antitoxin caused by intramuscular toxoid vaccination is immunoglobulin type gamma (IgG), the major immunoglobulin in the upper respiratory tract secretions of adult swine is immunoglobulin type alpha (IgA) (18). IgA is actively transported to mucosal surfaces and is of significant protective value against superficially replicating organisms (3), preventing adherence, mediating bacterial agglutination, and neutralizing certain toxins (15). IgG can reach nasal secretions by passive diffusion between epithelial cells and through exudation during inflammatory processes (3, 4, 15).

Intranasal instillation of PMT in piglets can cause severe snout lesions without causing a serologic response, as measured by serum neutralization tests (10). *P. multocida* bacterin-toxoid vaccination failures to prevent turbinate atrophy (6, 24) may be due to insufficient antibody levels on the nasal mucosal surface to inhibit colonization or neutralize PMT.

Beta-hydroxy, beta-methyl butyrate (HMB) is a naturally occurring product of leucine metabolism in sheep hepatocytes. Oral HMB supplementation in the piglet has been shown to stimulate the immune system by enhancing opsonization, stimulating B-lymphocyte blastogenesis and increasing macrophage activity (21).

The objectives of this study were to learn if there is a dose response effect of HMB on primary or anamnestic antibody levels following IM injection with a tetanus toxoid (primary), a killed Pseudorabies vaccine (anamnestic) and a *P. multocida* bacterin-toxoid vaccine (anamnestic). Immunoglobulin class specific anti-PMT titer was measured directly in nasal secretions and serum. To measure antibody titer on the nasal mucosa, a method of nasal secretion collection giving enough undiluted nasal mucosal surface secretions for antibody titration was devised. A commercially available anti-PMT ELISA was modified so immunoglobulin class specific titers in collected nasal secretions and sera could be measured.

Materials and Methods

Thirty-six Landrace by Large White cross gilts were randomly assigned to one of three consecutive time blocks, 12 gilts per block. Within each block, gilts were randomly assigned to one of 3 HMB treatments (0 gm, 2 gm or 10 gm) top-dressed on the feed once daily, beginning 38 days before the expected block farrowing date and continuing through 21 days post-farrowing. They were housed in groups of three, in separate isolation rooms before farrowing. Each gilt was fed separately four pounds of complete 18% protein corn and soybean based antibiotic free feed once daily before farrowing. Following farrowing, the same diet was fed ad libitum, ensuring all offered feed was eaten each day. They were farrowed in a common room containing 12 individual farrowing crates.

All gilts were injected intramuscularly in the neck with a commercially available killed pseudorabies (PRV) vaccine[a] and a tetanus toxoid[b] in separate sites. To test the effect of HMB treatment on immunoglobulin class specific humoral and mucosal antibody titers, gilts in each HMB treatment group were randomly assigned to be either *P. multocida* vaccinated or unvaccinated.

---

[a]PRV Gold®, Syntrovet Corp., Lenexa, KS

[b]Tetanus Toxoid®, Colorado Serum Corp., Fort Collins, CO

The *P. multocida* vaccinated gilts were injected according to label directions with a commercially available progressive atrophic rhinitis vaccine containing bacterins of *Bordetella bronchiseptica* and type D *Pasteurella multocida* along with a *P. multocida* toxoid.[c] The Tetanus toxoid and PRV vaccines were also given according to label directions. Each vaccine was given by individual injection into separate intramuscular sites using an 18 gauge, $1\frac{1}{2}$ inch long needle. Vaccination occurred on days −28 and −14, before the projected farrowing date for each block.

Sterile 13 mm plastic test tubes[d], sealed with rubber 100 ml. size bottle stoppers were used to collect nasal samples. A portable vacuum pump was connected to the test tube by rubber tubing attached to a 16 gauge, 1 inch long needle inserted through the rubber stopper. The intravenous end of an 18 gauge Vaccutainer[e] needle was also inserted through the rubber stopper. A six inch length of 20 gauge silastic tubing was pushed over the exposed end of the Vaccutainer needle. The free end of the tubing was plugged with hematocrit clay and three holes were cut in the side of the tubing just proximal to the clay plug. As vacuum was applied through the rubber tubing to the test tube, nasal secretions were drawn into the test tube and collected in its bottom.

---

[c]AR Vac®, NOBL Laboratories, Orange City, Ia.

[d]12 x 75 mm Falcon® tube, Becton Dickinson Labware, Lincoln Park, New Jersey

[e]Vaccutainer® needle, Iowa Vet Supply, Des Moines, Iowa

Gilts were restrained by putting a soft cotton rope in the mouth and around the snout, tying the rope to either a wall ring or farrowing crate. Before collection the head was secured in an upright position and the external nares were wiped with a gauze sponge. Nasal secretions were collected by vacuum from each nostril. If, at any time during the collection, any blood was visible in the tube or sample, the sample was discarded and collection was attempted the following day. Blood was collected from the cranial vena cava immediately following successful nasal sample collection.

Serum samples were collected on days –38 (before beginning HMB treatment), –28, –14, and –7 prefarrowing, and on days 1, 7, 14, and 21 postfarrowing. Collection of nasal samples was attempted on days 38 prefarrowing, and days 1, 7, 14 and 21, postfarrowing. If during collections a gilt resisted forcefully enough to endanger her well-being, the collection was stopped and attempted the next day.

Collected nasal sample volumes ranged from one to four milliliters. The samples were transferred to microcentrifuge tubes and EDTA, 100 mM, was added to the volume of nasal sample to reach a final concentration of 1 mM EDTA per sample. Sputolysin[f] was added to the sample so the final volume ratio of Sputolysin to sample was 1:200. The microcentrifuge tube was mixed on a Vortex mixer and allowed to stand, at room temperature, for 30 minutes. Following centrifugation at 1000

---

[f]Sputolysin Reagent®, Calbiochem-Novabiochem Corp., La Jolla, CA

RPM for 3 minutes the samples were frozen at -70° C. until analysis.

Serum was separated from the blood and transferred to labelled Falcon tubes. All samples were stored at –70° C. until analysis.

Concentrations of HMB were measured in the gilt sera according to the protocol of Nissen et al. (20)

Class specific antibody titration was done by a modification of the procedure used by Foged et al. (8) ELISA plates, with wells coated with monoclonal anti-PMT antibody, were supplied by DAKO Corporation, Carpenteria, California[g]. Serial dilutions of PMT, harvested from cultures of toxigenic *P. multocida*[h], according to Ackerman et al. (1), were assayed for activity. One hundred microliters of PMT, diluted 1:250, were incubated for 30 minutes, with shaking, in each ELISA well. Nonspecific antibody binding was blocked by shaking 100 μl of 1% mouse serum[i] in the wells for 30 minutes. Fifty microliters of appropriate sample serial dilutions, beginning at 1:2, were added to the wells and incubated, with shaking, for 1 hour. One hundred microliters of 1:1000 anti-IgA or anti-IgG class specific antibody conjugated with horse radish peroxidase[j] were added to each well for 1 hour, with shaking. Between these steps, wells

---

gDAKO PMT ELISA Kit®, DAKO Corporation, Carpenteria, California

hNational Animal Disease Lab, Ames, Iowa

iStellar Bio Systems, Inc., Columbia, MD

jBethyl Laboratories, Bethyl, TX

were washed four times with 200 μl phosphate buffered saline solution with 0.5% TWEEN. A substrate of 100 μl OPD solution prepared according to kit directions was added and incubated without shaking, in the dark, for 15 minutes. All incubations were at room temperature. Color development was stopped by the addition of 100 μl H_2SO_4 supplied with the ELISA kit and optical density was read by an automatic optical density reader at 490 nm. The titer was calculated using extrapolation between the log values of the end titer dilutions, according to directions supplied with the ELISA kits.

Nasal swabs were taken on each sampling day and streaked for bacterial isolation on both 5% sheep blood agar and *P. multocida* selective agar, prepared according to the procedure of Hoffman (13). Identification of individual colonies of *P. multocida* was done using standard biochemical tests. The DAKO PMT ELISA® test kit verified toxigenicity of the cultures.

Sera was submitted to the Iowa State University College of Veterinary Medicine Diagnostic Lab for G1 deleted differential pseudorabies titer determination. Anti-tetanus titers were performed using an ELISA, according to the procedure of Goff et al. (12)

Data were analyzed by the general linear models procedure of SAS using baseline serum titers as covariates for the Pseudorabies and Tetanus titers. The immunoglobulin class specific anti-PMT antibody titers were analyzed by the general linear models procedure of SAS, after subtracting the initial baseline titers from all results.

Results

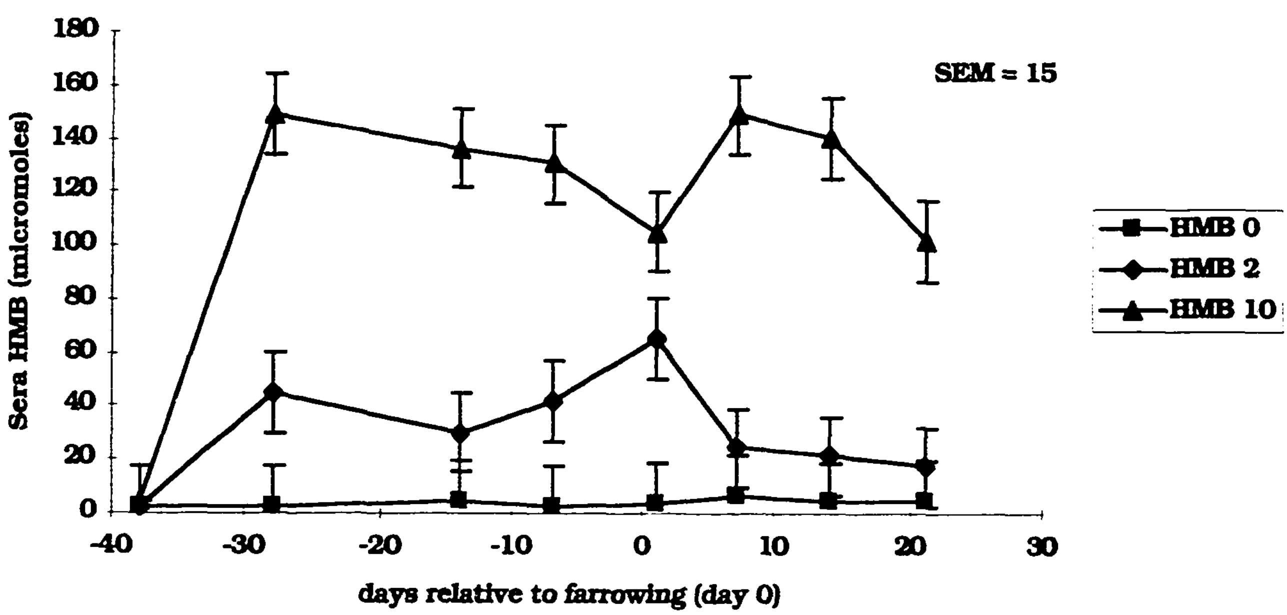
Cultures

No toxigenic *P. multocida* was isolated from the gilts during the trial.

Sera HMB

Sera concentrations of HMB are shown in Figure 1. All day values are relative to farrowing (day 0). Sera concentrations of HMB on day –38 were from samples taken before beginning dietary supplement with HMB and were not statistically unique in any of the treatment groups. Statistically significant differences among treatment groups were found during each of the subsequent sampling dates. Significance was at $p<0.01$ for each of these measurements.

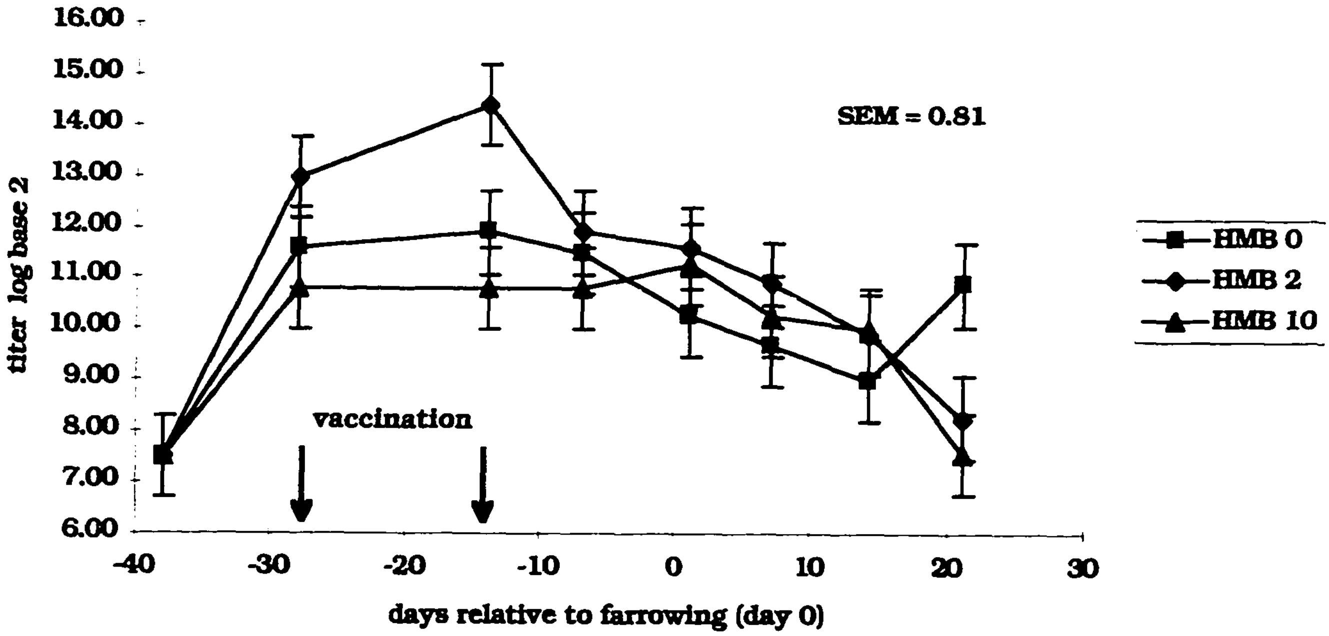
Figure 1. HMB sera levels in gilts fed varying levels of HMB top-dressed on the feed. For all points across treatments $p<0.01$.



Anti-tetanus titers

The sera anti-tetanus titers are detailed in Figure 2. The titer on day –38 was the baseline titer calculated by the covariate analysis.

Figure 2. HMB effect on sera anti-tetanus titers, log base 2, of gilts fed varying levels of HMB.

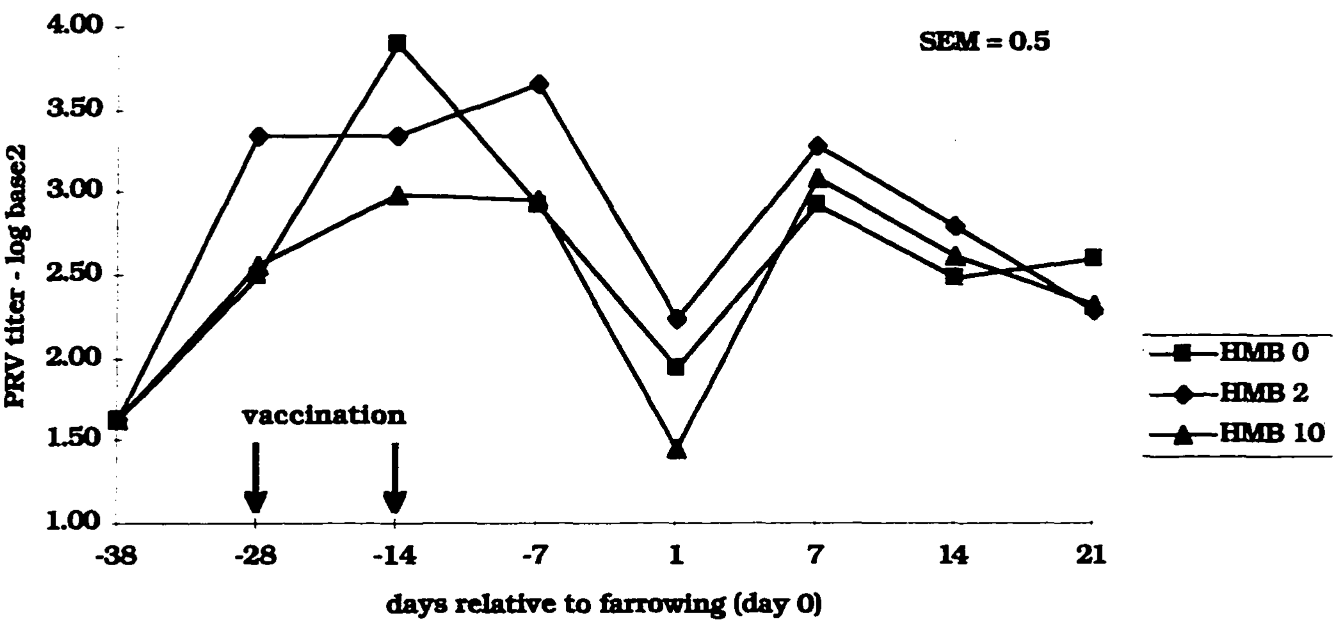


Samples taken on day –38 were done so before vaccination and dietary supplement with HMB.

Anti-pseudorabies virus titers

Figure 3 represents the log base 2 anti-PRV titers. All days are compared with farrowing (day 0). The titer on day –38 was the baseline titer calculated by the covariate analysis. Samples taken on day –38 were done so before vaccination and dietary supplement with HMB.

Figure 3. Sera anti-pseudorabies titers (log base 2) of gilts fed varying levels of HMB once daily.Class specific anti-PMT titers



Figures 4 through 11 show the class specific anti-PMT titers by day relative to farrowing (day 0) in the sera and nasal secretions as a function of vaccination and treatment with HMB. The nasal samples and their corresponding sera samples were collected on days –38, 1, 7, 14 and 21. All values were corrected by subtracting the respective baseline titer found on day -38. This allows statistical analysis of the data from a starting titer of zero on day –38.

Figure 4. Vaccination effect on sera anti-PMT IgA titers in gilts fed varying levels of HMB once daily (PMT vaccination on days -28 and -14).

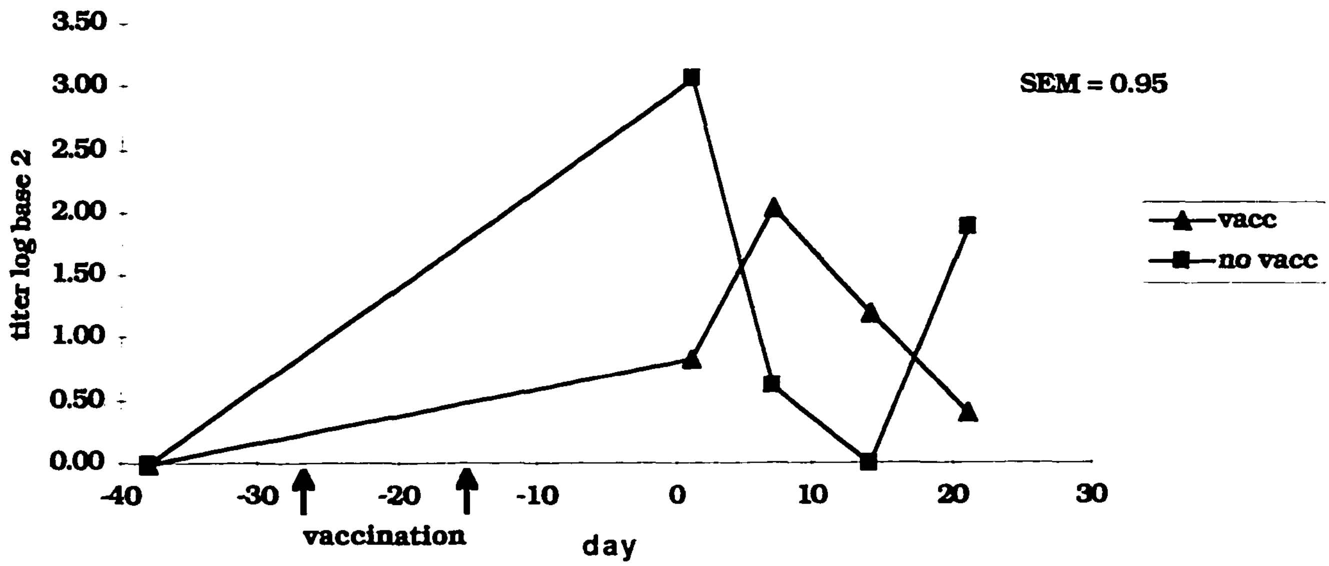


Figure 5. HMB effect on sera anti-PMT IgA titers in gilts fed varying levels of HMB once daily (PMT vaccination on days -28 and -14).

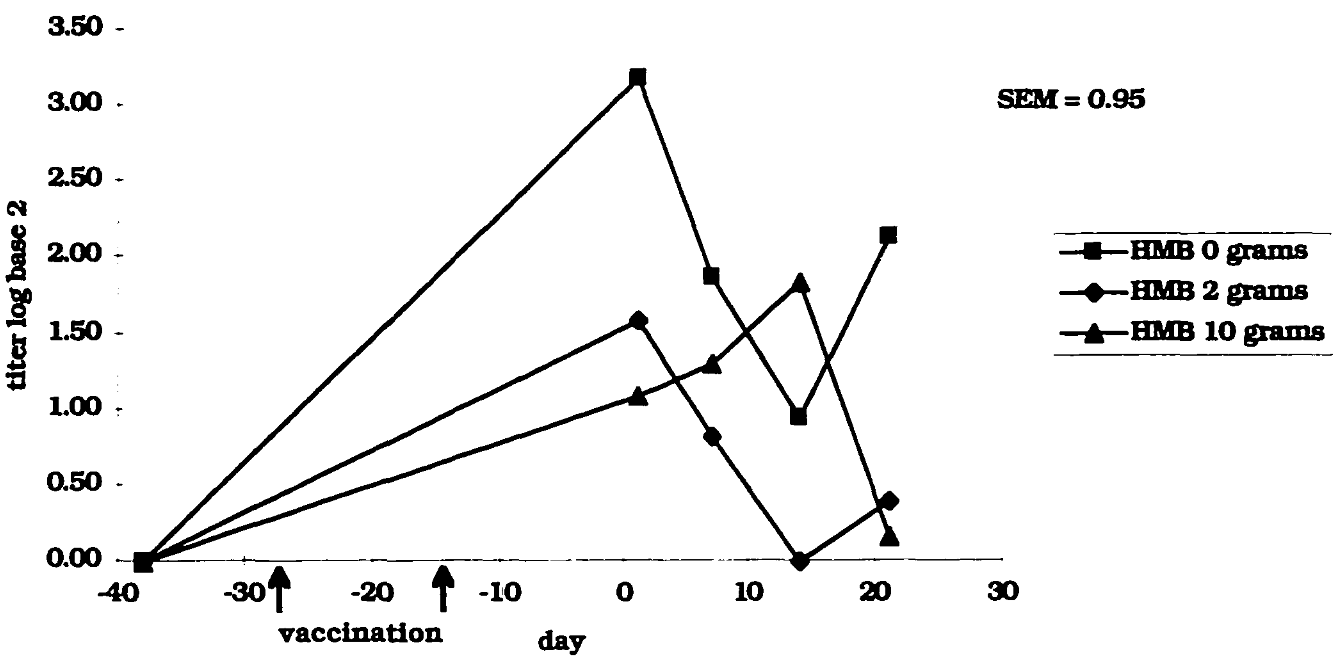


Figure 6. Vaccination effect on sera anti-PMT IgG titers in gilts fed varying levels of HMB once daily (vaccination on days –28 and –14).

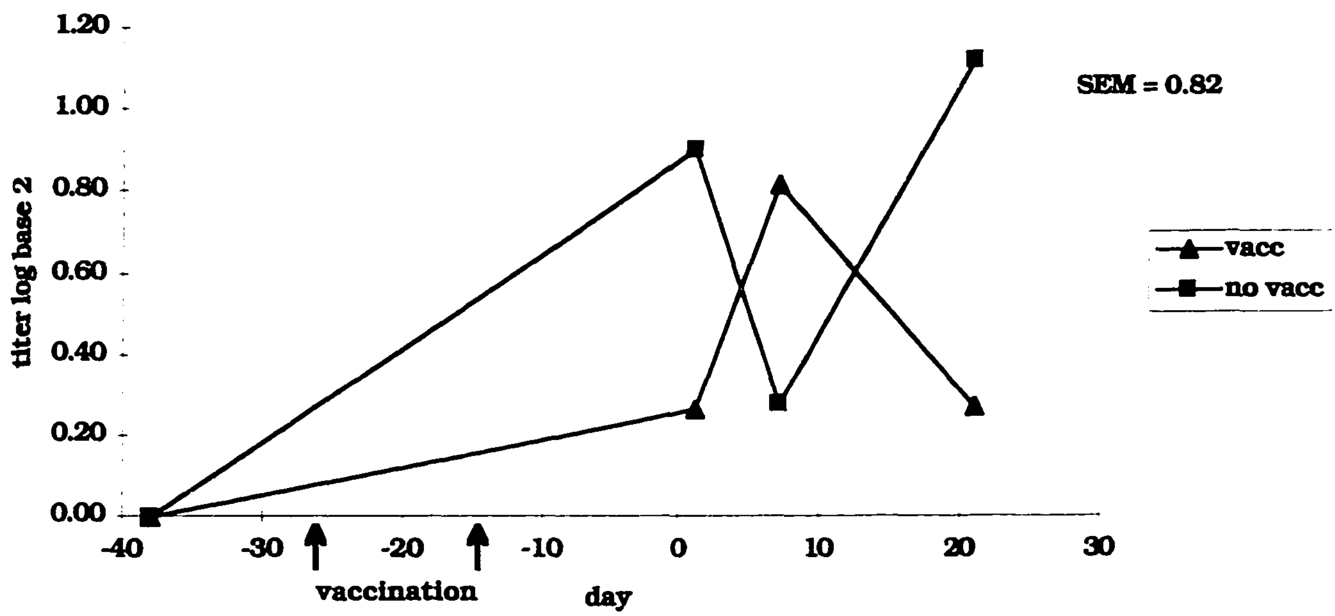


Figure 7. HMB effect on sera anti-PMT IgG titers in gilts fed varying levels of HMB once daily (PMT vaccination on days –28 and –14).

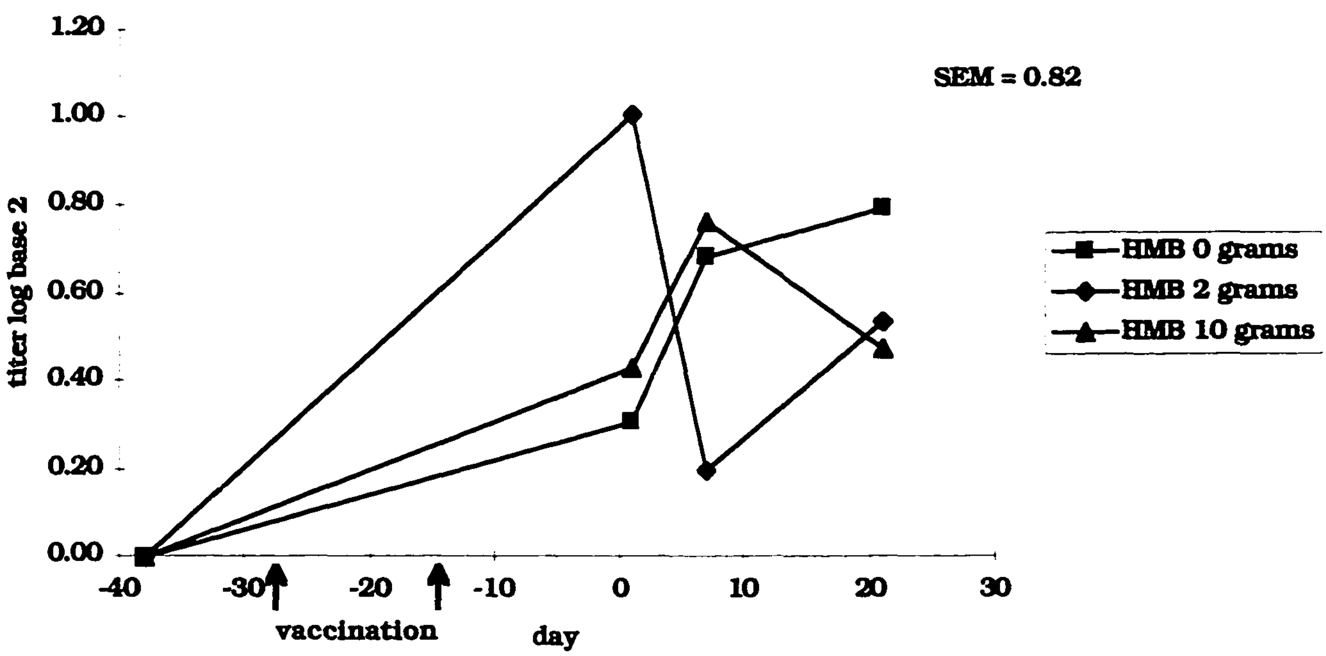


Figure 8. Vaccination effect on nasal anti-PMT IgA titers in gilts fed varying levels of HMB once daily (vaccination on days –28 and –14).

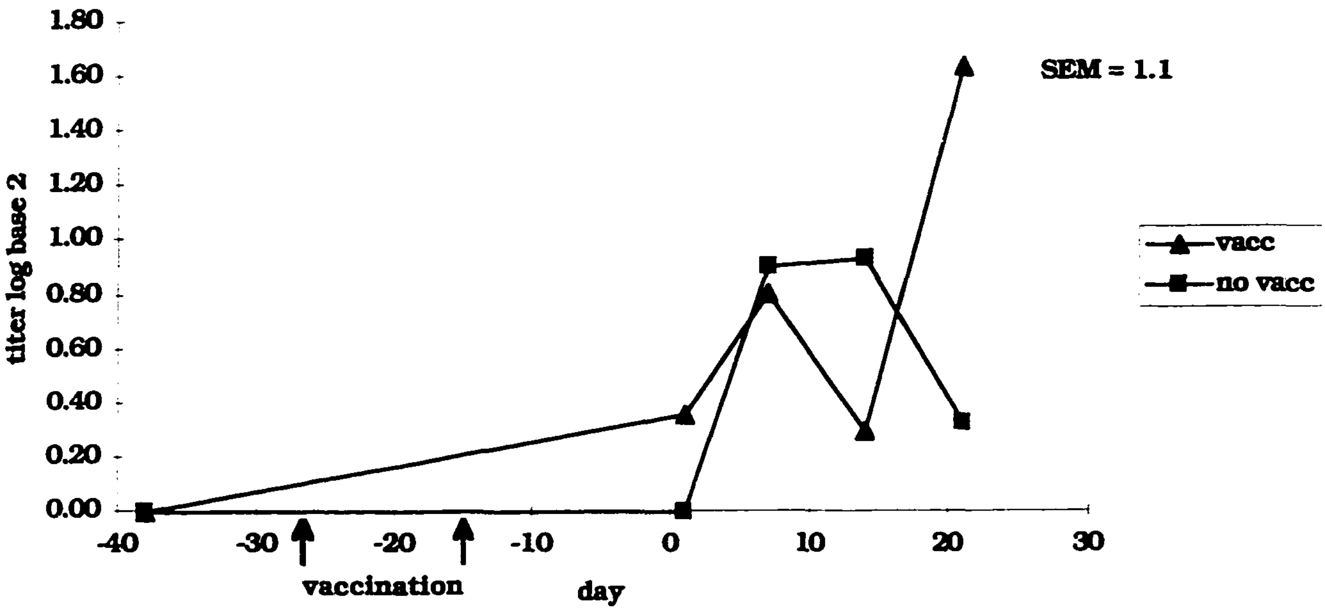


Figure 9. HMB effect on nasal anti-PMT IgA titers in gilts fed varying levels of HMB once daily (PMT vaccination on days –28 and –14).

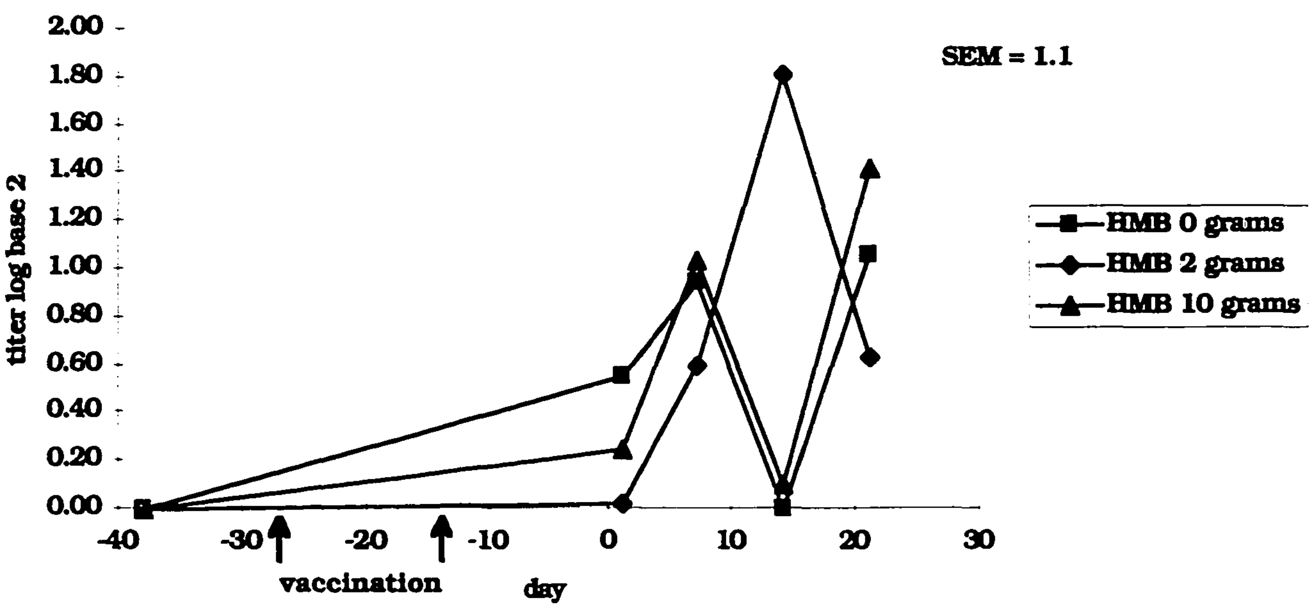


Figure 10. Vaccination effect on nasal anti-PMT IgG titers in gilts fed varying levels of HMB once daily (PMT vaccination on days –28 and –14).

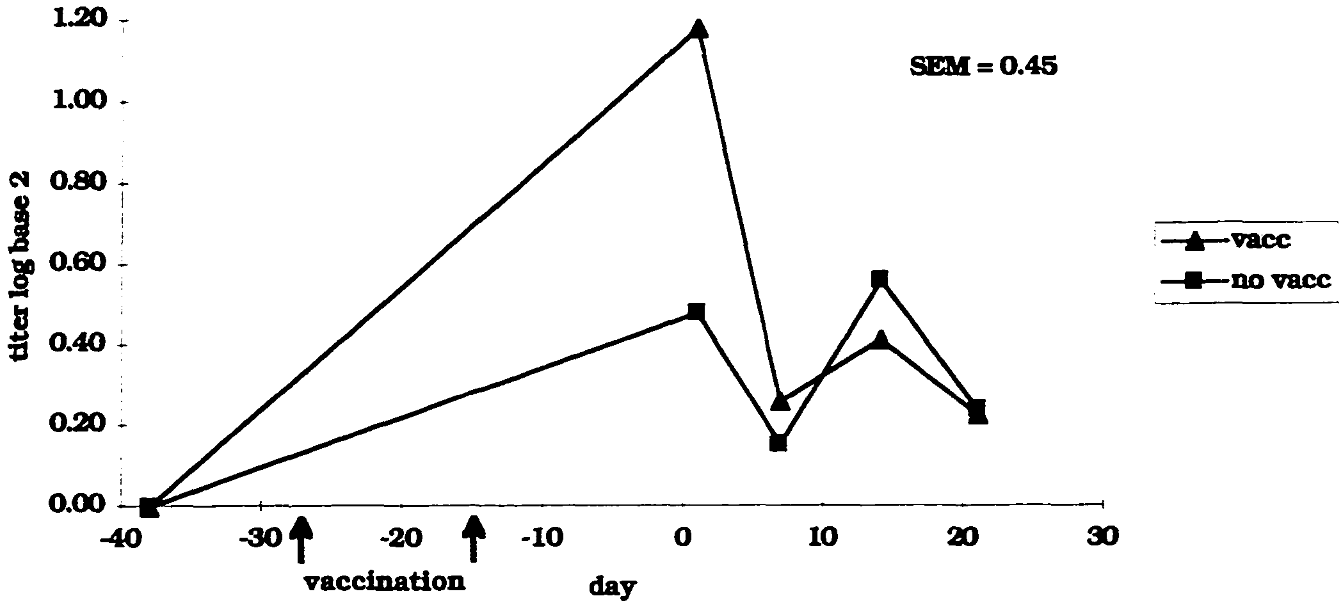
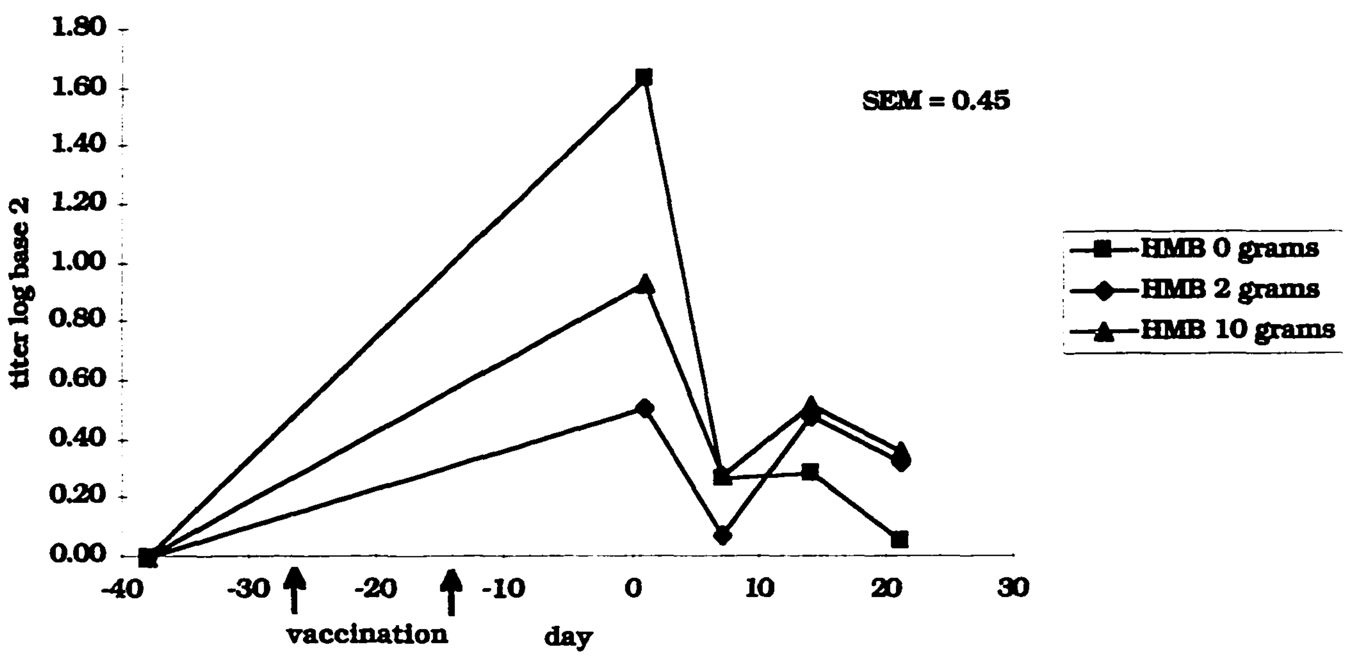


Figure 11. HMB effect on nasal anti-PMT IgG titers in gilts fed varying levels of HMB once daily (PMT vaccination on days –28 and –14).



Discussion

Today's pork production industry relies less on treatment of disease and more on prevention. Because of the economic consequences of disease treatment, more emphasis has been placed on disease prevention. Part of most pork producer's disease prevention strategy uses vaccines. Identifying a technology allowing the use of fewer animal health products is important to pork producers because of the potential for decreasing costs of production.

Beta-hydroxy, beta-methyl butyrate (HMB) has shown some promise to be an immune system stimulator. Heightening immune system status, particularly that of the mucosal surfaces, may give the pig protection from infectious agents that could affect the pig's growth performance. This study attempted to evaluate the usefulness of HMB as an immune system enhancer. The gilts were given their respective dose of HMB, top-dressed on their feed once daily, beginning approximately 38 days before farrowing. There was some variation in gestation lengths so an individual gilt may have received HMB for a longer or shorter time than 38 days. One gilt did farrow uneventfully 2 weeks before the date specified by her breeding date and she was removed from the study. All other gilts farrowed within a few days of the scheduled farrowing dates.

Table 1 shows the gilts successfully absorbed the fed HMB. There is a statistical difference at the $p<0.01$ in the sera levels of HMB between treatments. The drop in HMB level in the 10 gram treatment group at farrowing is not enough to change the $p<0.01$ probability.

A tetanus toxoid was included in the vaccination protocol as an attempt to use an immunogen that would give a primary immunological response. None of the gilts were serologically negative to tetanus toxin at the beginning of the study, so a primary response was not able to be evaluated. The specificity of the ELISA used in the anti-tetanus antibody assay had not been investigated sufficiently to rule out cross reaction with antibodies against Clostridial toxins a pig may routinely contact, such as from *Clostridium perfringens*.

The anti-tetanus titer in those pigs receiving 2 grams of HMB per day tended to be consistently higher than that of the other two groups. Vaccination was given on day –28, and by day –14 the 2 gram HMB treatment group had a statistically higher titer than either the 0 or 10 gram treatment groups. No anamnestic response to vaccination was seen after the day –14 vaccination.

A commercial swine herd was the source of the gilts. They had been vaccinated against pseudorabies at the time of breeding but were negative to anti-wild virus pseudorabies antibodies by the differential ELISA used in the Iowa State University College of Veterinary Medicine Diagnostic Laboratory at the beginning and throughout the study.

Except on day –14, PRV antibody titer in the 2 gram HMB treatment group consistently tended to have higher titers than either the 0 or 10 gram groups. On days –28 and –7 the difference between the 2 and 0 gram groups was statistically significant at the $p<0.10$ level. The high titer of the 0 gram gilts on day –14 is not explainable. The high value may be due to statistical variation, as there was no significant difference among the levels on this day. HMB did not affect the pseudorabies titers postfarrowing, other than on day 1, in which the gilts fed 10 grams HMB were significantly lower than those fed 2 grams.

HMB effect on class specific antibody production is variable and needs to be further studied to understand its effects. The serum levels of anti-PMT IgA appeared to have been suppressed by HMB supplementation (Figure 5). There was a significantly higher serum IgA level on day 21 in the gilts not fed HMB. Also, the trend in the serum appears to be for the non-supplemented pigs to have higher IgA levels than those supplemented. Serum anti-PMT IgG levels (Figure 7) were variable enough that no pattern emerges except at day 1, when the 2 gm HMB group had the highest titer.

Nasal anti-PMT IgA levels (Figure 9) were also variable, with no discernable differences among the HMB treatment groups. Nasal anti-PMT IgG levels, seen in Figure 11, varied but tended to be greater in pigs receiving no HMB on day 1, with no discernable differences on the following days.

Vaccination with the bacterin-toxoid combination didn't greatly affect sera or nasal class specific antibodies. There was a vaccine effect on sera anti-PMT IgA levels on days 1 and 21 (Figure 4) but the results are somewhat unexpected in that the titers of the non-vaccinated gilts were greater than those vaccinated. The proof of a vaccine is in clinical and economic response, but in this measurement vaccination did not appear effective.

The vaccination and HMB interaction was primarily unremarkable. There was some interaction with HMB on the levels of sera IgA, at day

21, and nasal IgA, at day 14, with unvaccinated gilts tending to have the higher titers. HMB supplementation in the non-vaccinates produced a higher titer on day 14 than that in the gilts receiving no HMB.

There is a downward movement in the anti-tetanus, anti-pseudorabies and sera and nasal anti-PMT titers within the first 2 weeks post-farrowing. This is most striking in the anti-pseudorabies titers (Figure 3). All gilts had a remarkable decrease in titer on day 1. Vaccination protocols are designed to maximize titer at farrowing. This drop was unexpected and may be related to movement of antibody into colostrum or parturition stress factors, but this needs clarification.

The apparent HMB inhibition of sera IgA response, which was not strongly statistically supported, is not well explained by this study. It conflicts with the increase in anti-tetanus and anti-pseudorabies caused by feeding 2 gm HMB, seen in Figures 2 and 3. Still, several possibilities other than statistical variation exist.

The immune response starts with an antigen presenting cell (APC) processing the antigen and presenting it, on its surface, to T and B cell lymphocytes. The T helper lymphocyte (T_h) is thought to then contact the presented antigen, release interleukins that stimulate B cell response and facilitate the interaction of the B cell with the presented antigen (22). HMB, when fed to chickens as 0.1% of their diet, has been shown to stimulate macrophage (an APC) activity and B cell response. For chickens receiving HMB at 0.05% of their diet, macrophage activity and lymphocyte response was depressed below control levels (21). It is possible HMB has a depressing activity on antigen presentation or B cell activity when it is fed below a certain threshold level. The work in the chicken does not address what effect supplementing a percentage of the diet higher than 0.1% may have.

The pigs in this study were given four pounds of feed daily during gestation, then fed ad libitum post-farrowing. Ten grams of HMB supplemented once daily would account for approximately 0.1% of the daily intake during gestation. Further testing is required to be able to state whether or not HMB at 0.1% of the diet in the pig is inhibitory or stimulatory. With two grams of HMB (approximately 0.02% of the pre-farrowing diet) appearing to stimulate the immune response to pseudorabies and tetanus toxoid, it is possible a certain intake of HMB may be stimulatory to antigen presentation or B cell reaction to specific antigens and not to others.

Another potential effect of HMB involves immune response differentiation. Comparing Figures 5 and 7 shows at least an initial preference for production of IgG when treated with HMB. Differentiation and maturation of the immune response is influenced by T_h cells with a $CD4^+$ phenotype releasing lymphokines that help coordinate the immune function (22). HMB at specific levels in the diet may have an influence on the isotype of antibody produced during an immune reaction by affecting the type or ratio of lymphokines released. The anti-tetanus and anti-pseudorabies titers measured total antibody titer, which may have masked a preference to one antibody isotype.

Many factors are involved in transmission of *P. multocida* and the occurrence of clinical progressive atrophic rhinitis. Vaccination programs have been one practice pork producers have used to help prevent the physical and economic effects of the condition. In this study, the vaccine used did not appear to greatly effect the level of anti-PMT antibodies in the nasal secretions or the sera. Further study, designed to evaluate the protective response of vaccination to exposure, could help determine the vaccine's effectiveness.

Bibliography

1. Ackermann, M.R., R.B. Rimler, J.R. Thurston. 1991. Experimental model of atrophic rhinitis in gnotobiotic pigs. Infection and Immunity. **59**: 3626-3629.

2. Baalsrud, K.J. 1987. Vaccination against atrophic rhinitis: effect on clinical symptoms, growth rate and turbinate atrophy. Acta Vet Scand. **28**: 305-311.

3. Berstein, J.M. 1992. Mucosal immunology of the upper respiratory tract. Respiration. **59 (suppl. 3)**: 3-13.

4. Brandtzaeg, P. 1984. Immune functions of human nasal mucosa and tonsils in health and disease. p. 28-95. **Immunology of the lung and upper respiratory tract**. J. Bienenstock, ed. McGraw-Hill Book Co., New York.

5. Chanter, N., J.M. Rutter, and A. Mackenzie. 1986. Partial purification of an osteolytic toxin from *Pasteurella multocida*. J Gen Micro. **132**: 1089-1097.

6. Chanter, N. and J.M. Rutter. Atrophic rhinitis. pp 162-195. **Pasteurella and Pasteurellosis.** C. Adlam and J.M. Rutter, eds. Academic Press, London.

7. Chanter, N. and J.M. Rutter. 1990. Colonization of *Pasteurella multocida* in atrophic rhinitis of pigs and immunity to the osteolytic toxin. Vet Micro. **25**: 253-265.

8. Foged, N.T., J.P. Nielsen, and K. Barfod. 1990. The use of ELISA determination of *Pasteurella multocida* toxin antibodies in the control of progressive atrophic rhinitis. Proceedings 11th Int Pig Vet Soc Congr, Lausanne. p 49.

9. Foged, N.T., K.B. Pedersen, and F. Elling. 1987. Characterization and biological effects of the *Pasteurella multocida* toxin. FEMS Micro Letters. **43**: 45-51.

10. Frymus, T., E. Müller, B. Franz, and K. Petzoldt. 1989. Protection by toxoid-induced antibody of gnotobiotic piglets challenged with the dermonecrotic toxin of *Pasteurella multocida* . J Am Vet Med Assoc. **36**: 674-680.

11. Fuller, J.C. 1995. Personal communication. Metabolic Technologies, Inc., Ames, Iowa.

12. Goff, B.L., K.P. Flaming, D.E. Frank, E.R. Clough and J.A. Roth. 1991. Recombinant porcine somatotropin: An immunotoxicology study. J Anim Sci. **69**: 4523-4531

13. Hoffman, L.J. 1992. Personal communication. Iowa State University. Ames, Iowa.

14. Kabay, M.J., A.R. Mercy, J.M. Lloyd, and G.M. Robertson. 1992. Vaccine efficacy for reducing turbinate atrophy and improving growth rate in piggeries with endemic atrophic rhinitis. Aust Vet J. **69**: 101-103.

15. Kaltreider, H.B. 1984. Local Immunity. pp 191-215. **Immunology of the lung and upper respiratory tract**. J. Bienenstock,ed. McGraw-Hill Book Co., New York.

16. Martineau-Doizé, B., J. Frantz, and G. Martineau. 1990. Effects of purified *Pasteurella multocida* dermonecrotoxin on cartilage and bone of the nasal ventral conchae of the piglet. Anat Rec. **228**: 237-246.

17. Martineau-Doizé. B., J. Menard, C. Girard, J.C. Frantz, and G.P. Martineau. 1991. Effects of purified *Pasteurella multocida* dermonecrotic toxin on the nasal ventral turbinates of fattening pigs: histological observations. Can J Vet Res. **55**: 377-379.

18. Morgan, K.L. and F.J. Bourne. 1980. Immunoglobulin levels in porcine nasal and tracheal secretions - the influence of the method of collection. J of Immun Meth. **37**: 165-173.

19. Nielsen, J.P., N.T. Foged, V. Sorense, K. Barfod, A. Bording, and S.K. Petersen. 1991. Vaccination against progressive atrophic rhinitis with a recombinant *Pasteurella multocida* toxin derivative. Can J Vet Res. **55**: 128-138.

20. Nissen, et al. 1990. Anal. Biochem. **188**: 17-19.

21. Nissen, Steven. 1995. Personal communication. Iowa State University. Ames, Iowa.

22. Okuda, M. and R. Powankar. 1992. Flow cytometric analysis of intraepithelial lymphocytes in the human nasal mucosa. Allergy. **47**: 255-259.

23. Pedersen, K.B. and K. Barfod. 1984. Influence of vaccination of sows with *Bordetella-Pasteurella* vaccines on the occurrence of atrophic rhinitis among their offspring after experimental infection with *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida*. Nord Vet Med. **36**: 337-345.

24. Rutter, J.M. 1983. Virulence of *Pasteurella multocida* in gnotobiotic pigs infected with *Bordetella bronchiseptica*. Res in Vet Sci. **34**: 287-295.

25. Rutter, J.M. and A. Mackenzie. 1984. Pathogenesis of atrophic rhinitis in pigs: a new perspective. Vet Rec. **114**: 89-90.

IV. SYSTEMIC AND LOCAL ANTIBODY RESPONSE IN THE PIG AS AN INDICATION OF TOXIGENIC *PASTEURELLA MULTOCIDA* TYPE D TRANSMISSION

A paper prepared for the journal Infection and Immunity

Paul L. Sundberg, Eldon K. Uhlenhopp
and Lorraine J. Hoffman

Abstract

Twenty crossbred barrows and gilts six weeks of age were housed in groups of five and randomly assigned according to a 2x2 factorial design with treatments being vaccination and experimental intranasal exposure with a toxigenic strain of type D *Pasteurella multocida*. The vaccinated pigs were injected with a commercially available progressive atrophic rhinitis vaccine consisting of *Bordetella bronchiseptica* and *P. multocida* bacterins with a *P. multocida* toxin (PMT) toxoid. Vaccination preceded experimental exposure by 7 days.

Nasal secretions and serum samples were collected and assayed for class specific anti-PMT antibodies. Nasal secretion samples were collected before experimental exposure and 35 days later. Blood was collected by anterior vena cava venipuncture on days 0, 7, 21, 35, 98 and 124.

Nasal swabs were taken on days 0, 7, 21, 35 and 70 and streaked for bacterial isolation. Identification of individual colonies of *P. multocida* was performed using standard biochemical tests. A sandwich ELISA verified toxigenicity of the cultures.

The pigs were examined at slaughter for evidence of nasal turbinate

damage consistent with progressive atrophic rhinitis and lung consolidation.

Recovery of the organism on each sampling day, from even the experimentally exposed pigs, was not consistent. Only 6 of the 10 experimentally exposed pigs yielded recoverable *P. multocida* during the trial. Transmission to non-experimentally exposed pigs was demonstrated by culture as early as 7 days post experimental exposure with infection continuing as late as day 70.

No vaccinated, non-experimentally exposed pigs were culture positive on any of the sampling dates suggesting protection from transmission by vaccination. Vaccinated, non-experimentally exposed pigs were less culturable on day 7 ($p<0.1$) than were vaccinated and experimentally exposed pigs.

The most nasal turbinate damage tended to be in the pigs that were neither vaccinated nor experimentally exposed. The lowest scores tended to be in the pigs that, although they were experimentally exposed, were also vaccinated.

Anti-PMT IgG in the sera appears to be a good indicator of exposure, supporting the hypothesis that the non-experimentally exposed pigs became exposed by transmission within 21 days. Anti-PMT serum IgG tended to be highest in vaccinated pigs. Serum anti-PMT IgA does not appear to be as sensitive an indicator of exposure or vaccination.

Nasal secretion anti-PMT IgA can be used as an indication of transmission. Nasal titers for both the experimentally exposed and nonexposed groups tended to move together, indicating transmission.

It appears there are different abilities of all of these measurements to detect transmission. Turbinate damage is the least specific indication and does not correspond well with other methods. Culture results and serological and mucosal assay do support each other. The exposed pigs were more likely to be culture positive on day 7 and they had a high anti-PMT IgG titer by that day. Antibody assay and culture results suggested transmission had occurred. Finally, culture and antibody titer implied a protective effect from vaccination.

Introduction

Mucosal antibody responses after infection or vaccination are highly similar. The mucosal immune system in the eyes, upper respiratory tracts, and upper alimentary tracts of pigs apparently react, immunologically, as a whole (13).

Morgan and Bourne measured class specific antibodies collected by nasal lavage from piglets up to 10 weeks of age (16). They found the major change that occurs in the immunoglobulin content of respiratory tract secretions of piglets between 2 and 10 weeks of age is an increase in the concentration of IgA due to an increase in mucosal IgA synthesis. While the major immunoglobulin in the upper respiratory tract secretions of adult swine is IgA (15), IgG can reach nasal secretions by passive diffusion or leakage, particularly in inflamed mucosa (2).

Progressive atrophic rhinitis (PAR) is caused by the increase in osteoclastic activity and decrease in osteoblastic activity in the nasal turbinates after exposure to the toxin of *Pasteurella multocida* (PMT), independent from an inflammatory process (8). Intramuscular, intravenous, intraperitoneal, subcutaneous, (5, 14, 17, 18) and intranasal (6, 8) administration of PMT have caused turbinate lesions associated with PAR.

It is possible intranasal infection with *P. multocida* may not cause an immunological response to the toxin. PMT repeatedly instilled intranasally did not cause a serologic response, as measured by the serum neutralization test in mice, although severe snout lesions were found (9). Pigs naturally infected with large numbers of toxigenic *P. multocida* in the nasal cavity did not develop a detectable serological antitoxin antibody response when measured by toxin neutralization assays (3).

Intramuscular administration of PMT, however, has been shown to result in a powerful antibody response that may be protective (9). Anti-PMT antibodies have protected piglets challenged by intramuscular injection of PMT (9) or by experimental intranasal infection with toxigenic *P. multocida* type D (4).

Intramuscular injection bypasses the nasal mucosal barrier and experimental intranasal infection causes artificial irritation of the nasal mucosa. Either way, these sources of PMT offer the opportunity for serum derived IgG to play a role in toxin neutralization on the nasal mucosal surface.

This study measured the class specific anti-PMT antibody titer in the serum and directly in nasal secretions to attempt to detect transmission of toxigenic *P. multocida*. Direct collection of nasal secretions was used to ensure antibody titers were located on the nasal mucosa and could not have been accidentally washed into the collection from the oro-pharyngeal cavity, as could happen with some nasal lavage techniques. Culture of nasal swabs and development of an anti-PMT antibody titer in the nasal secretions or in serum were compared as methods to detect transmission. The effect of vaccination on local and systemic anti-PMT antibody levels, *P. multocida* colonization, and development of nasal turbinate degeneration were also studied.

Materials and Methods

Twenty crossbred barrows and gilts six weeks of age were randomly assigned to one of four isolation rooms, five pigs in each room. They were fed ad libitum an 18% protein, antibiotic free diet.

Within each room, pigs were randomly assigned according to a 2x2 factorial design with treatments being vaccination and experimental intranasal exposure[a]. Those pigs to be vaccinated were injected intramuscularly with a commercially available progressive atrophic rhinitis vaccine consisting of *Bordetella bronchiseptica* and *Pasteurella multocida* bacterins with a PMT toxoid.[b] Non-vaccinated pigs were injected intramuscularly with sterile saline. Vaccination preceded experimental exposure by 7 days.

---

[a]Toxigenic P. multocida strain number P4148, National Animal Disease Laboratory, Ames, Iowa

[b]AR Vac, NOBL Laboratories, Orange City, IA

Experimental exposure was by the method of Ackerman et al. (1) Pigs in the non-experimentally exposed group were instilled intranasally with sterile media identical to that used to grow the toxigenic *P. multocida* inoculum.

Sterile 13 mm plastic test tubes[c], sealed with rubber 100 ml. size bottle stoppers were used to collect nasal secretion samples. A portable vacuum pump was connected to the test tube by rubber tubing attached to a 16 gauge. 1 inch long needle inserted through the rubber stopper. The intravenous end of an 18 gauge Vaccutainer[d] needle was also inserted through the rubber stopper. A six inch length of 20 gauge silastic tubing was pushed over the exposed end of the Vaccutainer needle. The free end of the tubing was plugged with hematocrit clay and three holes were cut in the side of the tubing just proximal to the clay plug. As vacuum was applied through the rubber tubing to the test tube, nasal secretions were drawn into the test tube and collected in its bottom.

Nasal secretion samples were collected before experimental exposure and 35 days later. Sample volumes ranged from one to four milliliters. The samples were transferred to microcentrifuge tubes and EDTA, 100 mM, was added to the volume of nasal sample to reach a final concentration of 1 mM EDTA per sample. Sputolysin[e] was added to the

---

[c]12 x 75 mm Falcon® tube, Becton Dickinson Labware, Lincoln Park, New Jersey

[d]Vaccutainer® needle, Iowa Vet Supply, Des Moines, Iowa

[e]Sputolysin Reagent®, Calbiochem-Novabiochem Corp., La Jolla, CA

sample so the final volume ratio of Sputolysin to sample was 1:200. The microcentrifuge tube was mixed on a Vortex mixer and allowed to stand, at room temperature, for 30 minutes. Following centrifugation at 1000 RPM for 3 minutes the samples were frozen at -70° C. until analysis.

Blood was collected by anterior vena cava venipuncture on days 0, 7, 21, 35, 98 and 124 and the serum was separated and saved. All samples were frozen at -20° C. until assayed.

Class specific antibody titration was done by a modification of the procedure used by Foged et al. (7) ELISA plates, with wells coated with monoclonal anti-PMT antibody, were supplied by DAKO Corporation, Carpenteria, California[f]. Serial dilutions of PMT, harvested from cultures of toxigenic *P. multocida*[g], according to Ackerman et al. (1), were assayed for activity. One hundred microliters of PMT, diluted 1:250, were incubated for 30 minutes, with shaking, in each ELISA well. Nonspecific antibody binding was blocked by shaking 100 μl of 1% mouse serum[h] in the wells for 30 minutes. Fifty microliters of appropriate sample serial dilutions, beginning at 1:2, were added to the wells and incubated, with shaking, for 1 hour. One hundred microliters of 1:1000 anti-IgA or anti-IgG class specific antibody conjugated with horse radish peroxidase[i] were

---

[f]DAKO PMT ELISA Kit®, DAKO Corporation, Carpenteria, California

[g]National Animal Disease Lab, Ames, Iowa

[h]Stellar Bio Systems, Inc., Columbia, MD

[i]Bethyl Laboratories, Bethyl, TX

added to each well for 1 hour, with shaking. Between these steps, wells were washed four times with 200 µl phosphate buffered saline solution with 0.5% TWEEN. A substrate of 100 µl OPD solution prepared according to kit directions was added and incubated without shaking, in the dark, for 15 minutes. All incubations were at room temperature. Color development was stopped by the addition of 100 µl H_2SO_4 supplied with the ELISA kit and optical density was read by an automatic optical density reader at 490 nm. The titer was calculated using extrapolation between the log values of the end titer dilutions, according to directions supplied with the ELISA kits.

Nasal swabs were taken on days 0, 7, 21, 35 and 70 and streaked for bacterial isolation on both 5% sheep blood agar and *P. multocida* selective agar that was prepared according to the procedure of Hoffman (12). Identification of individual colonies of *P. multocida* was performed using standard biochemical tests. The DAKO PMT ELISA® test kit verified toxigenicity of the cultures.

The pigs were examined at slaughter for evidence of lung consolidation and nasal turbinate damage consistent with progressive atrophic rhinitis. The space between the ventral aspect of the turbinate scrolls and the ventral floor of the nasal passage was measured in millimeters. This measurement was then compared to the values in Table 1 to assign a rhinitis score. In this system a space of 3 to 6 mm is considered normal, with greater or lesser values indicating PAR pathology.

Table 1. Scale for Scoring Turbinate Atrophy

Space between turbinates and nasal passage floor (mm)	Score	Interpretation
0 to 2	1	negative
3 to 6	0	negative
7 to 9	1	negative
10 to 12	2	suspect
13 to 16	3	mild atrophy
17 to 20	4	moderate atrophy
21 or more	5	severe atrophy

Modified from TRAC slaughter check form, Elanco Animal Health, Indianapolis, Indiana

Serum and nasal immunoglobulin data were analyzed using the general linear models procedure of SAS with baseline titers as covariates. Other data were analyzed using the SAS general linear models procedure.

Results

Culture

Table 2 shows only one of the ten non-experimentally exposed pigs, pig number 235 in room 10, cultured positive for toxigenic *P. multocida* at the first sampling date which was 7 days after contact with experimentally exposed cohorts. Six of the ten experimentally exposed pigs had a positive culture at least once during the sampling period, as early as day 7 and as late as day 70. No pigs that were vaccinated and non-experimentally exposed were culture positive during the study.

Nasal cultures were given a value of one if toxigenic *P. multocida* was isolated and zero if it was not. Statistical analysis is shown in Table 3.

Table 2. Identification, vaccination and experimental exposure status and culture results of pigs experimentally and non-experimentally exposed to toxigenic *P. multocida*.

				Days Post Experimental Exposure							
Room	ID	Vacc	Exper Exposure	0	7	14	21	28	35	42	70
7	231	+	–	–	–	–	–	–	–	–	–
7	232	+	+	–	+	+	+	+	+	+	–
7	233	–	+	–	+	+		+	+	+	–
7	245	+	+	–	+	–	–	–	–	–	–
7	247	–	–	–	–	+	+	–	–	+	+
8	230	+	–	–	–	–	–	–	–	–	–
8	241	–	+	–	–	–	–	–	–	–	–
8	242	+	+	–	–	+	–	–	–	–	–
8	244	–	+	–	–	–	–	–	–	–	–
8	248	–	–	–	–	–	–	–	–	–	–
9	237	+	+	–	–	–	–	–	–	–	+
9	239	–	–	–	–	–	–	–	–	–	–
9	243	+	–	–	–	–	–	–	–	–	–
9	246	–	+	–	–	–	–	–	–	–	–
9	249	–	–	–	–	–	–	–	–	–	–
10	234	–	+	–	–	–	–	–	–	–	–
10	235	–	–	–	+	+	–	–	+	+	–
10	236	+	+	–	–	+	+	–	–	–	+
10	238	+	–	–	–	–	–	–	–	–	–
10	240	+	–	–	–	–	–	–	–	–	–

Table 3. Numerical scoring of culture results from nasal swabbing of pigs, by treatment group; 0 = culture negative, 1 = culture positive.

Treatment Group	Days Post Experimental Exposure				
	0	7	21	35	70
not vacc, non-exp exposed	0	0.4	0.0	0.4	0.2
not vacc, exp exposed	0	0.2	0.2	0.2	0.0
vacc, non-exp exposed	0	0.0^{A}	0.0	0.0	0.0
vacc, exp exposed	0	0.6^{A}	0.2	0.2	0.4

$^{A}p<0.1$; Ave SEM = 0.2

There was a lower recoverability on day 7 from those pigs vaccinated and non-experimentally exposed when compared to the vaccinated and experimentally exposed pigs. This was the only statistically significant difference among any of the treatment groups.

Rhinitis scores and lung examination

Table 4 shows the mean rhinitis score for each treatment group. The not vaccinated, non-experimentally exposed pigs had a higher mean rhinitis score than either the not vaccinated, experimentally exposed or the vaccinated, experimentally exposed pigs, with statistical significance at the p=0.15 and p=0.13 levels, respectively.

Table 4. Mean rhinitis scores of pigs by treatment group.

		Exp Exposure		
		yes	no	MEAN
VACC	yes	2.4^{B}	2.8	2.6
	no	2.7^{A}	$3.8^{A,B}$	3.3
	MEAN	2.6	3.3	

$^{A}p=0.15$, $^{B}p=0.13$; Ave SEM = 0.5

No gross lung lesions were found in any of the pigs after slaughter.

anti-PMT antibody in the nasal secretions and serum

The log base 2 class specific anti-PMT antibody titers of the sera are shown in Figures 1 through 4. Figure 1 shows the anti-PMT IgG level as affected by exposure. There was an initial increase in serum anti-PMT IgG in both the experimentally and non-experimentally exposed groups. The rise in titer in the experimentally exposed pigs occurred by day 7 post-experimental exposure, then tended to level off through day 128. The non-experimentally exposed pigs had a later titer increase (day 21 after inoculation of the experimentally exposed pigs), leveled off through day 70 then began a steady increase through day 126. Figure 2 shows the anti-PMT IgG level as affected by vaccination. The vaccinated and non-vaccinated pigs both increased in titer, roughly parallelling each other until day 128.

Experimental exposure had no statistical effect on serum anti-PMT IgA but, there was a trend for the serum anti-PMT IgA in the experimentally exposed group to increase, post-exposure, while decreasing in the non-experimentally exposed pigs (Figure 3). By day 72, the non-experimentally exposed group had increased serum anti-PMT IgA to roughly the same level as the other group. The serum anti-PMT IgA levels as a function of vaccination (Figure 4) were approximately the same through day 21, post-exposure, then the non-vaccinated group tended to have a parallel but higher titer than the vaccinated pigs.

Figure 1. Effect of exposure to toxigenic *P. multocida* on sera anti-PMT IgG (log base 2) in pigs.

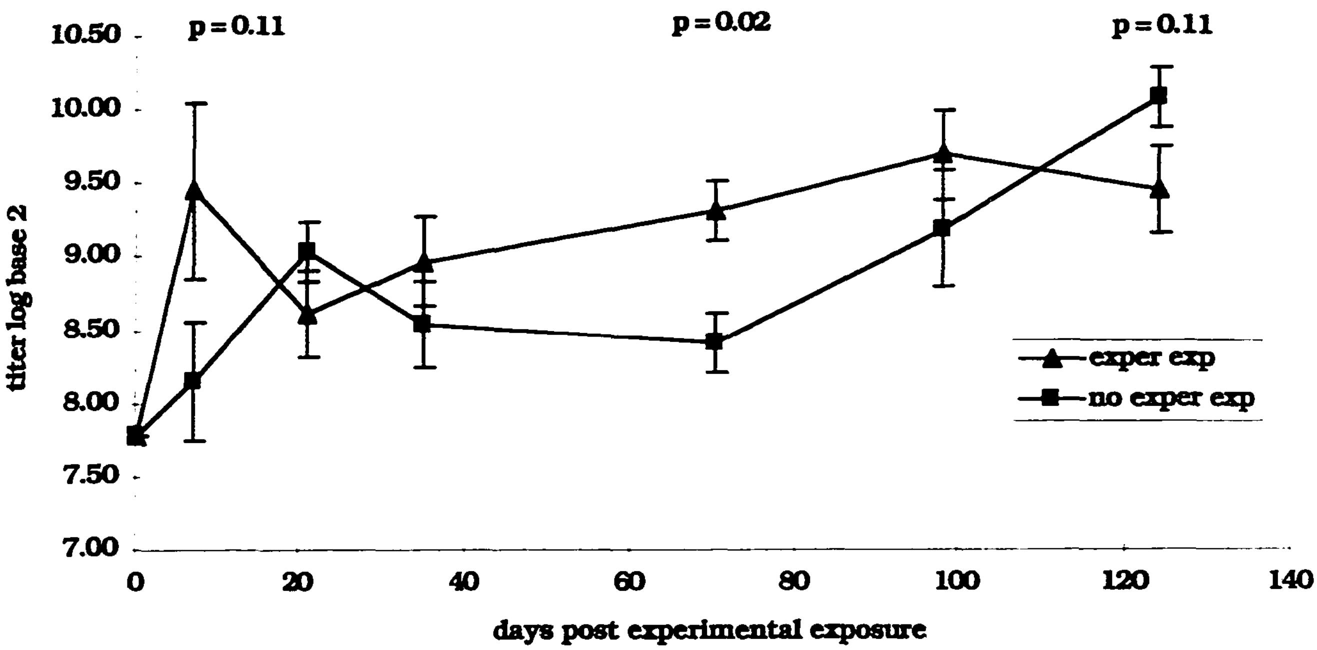


Figure 2. Effect of vaccination with a bacterin/toxoid atrophic rhinitis vaccine on sera anti-PMT IgG (log base 2) in pigs.

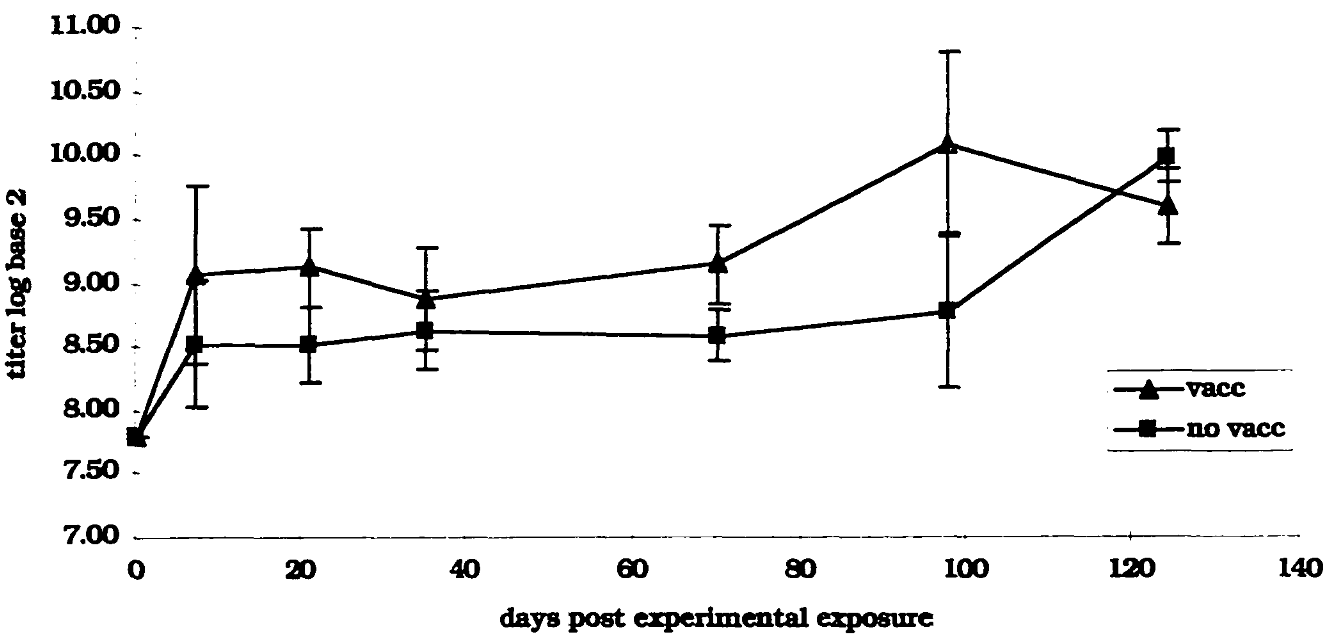


Figure 3. Effect of exposure to toxigenic *P. multocida* on sera anti-PMT IgA (log base 2) in pigs.

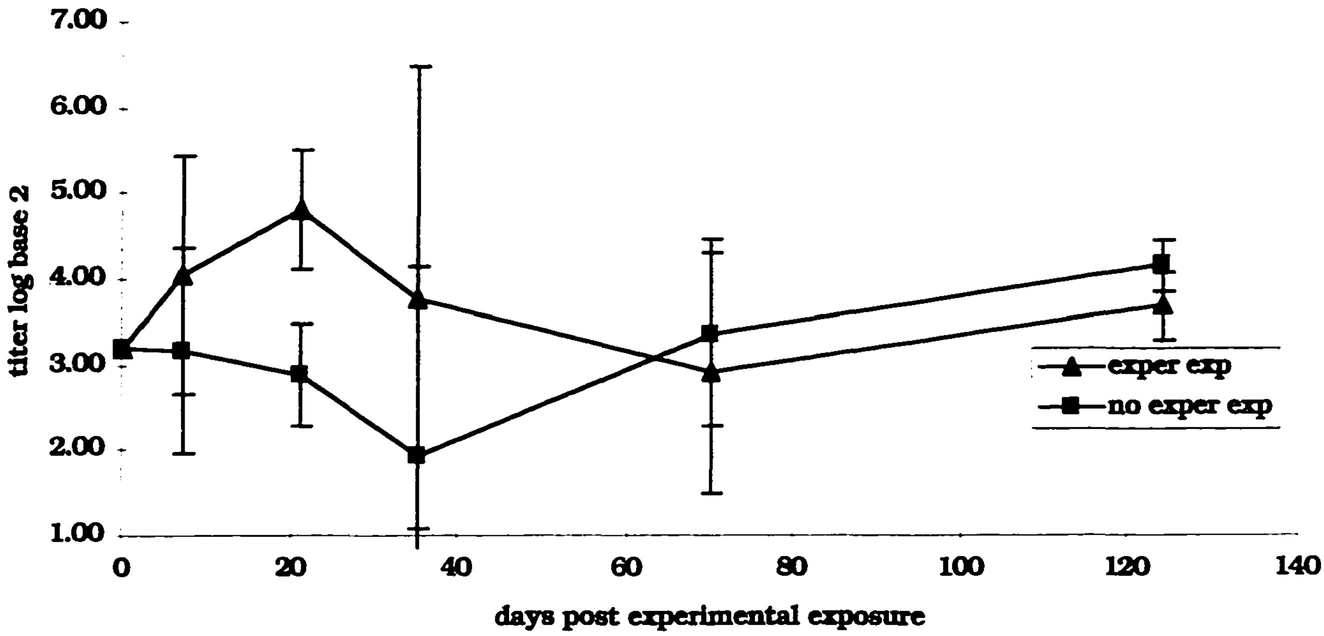
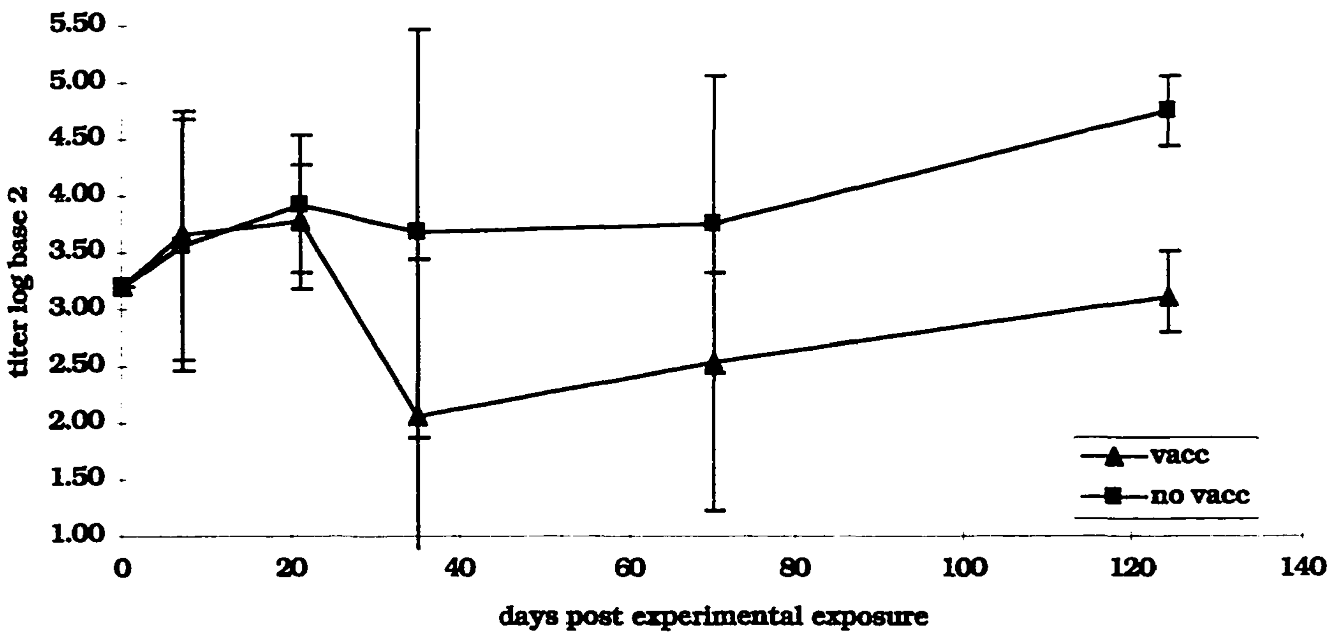


Figure 4. Effect of vaccination with a bacterin/toxoid atrophic rhinitis vaccine on sera anti-PMT IgA (log base 2) in pigs.



Care was taken during nasal sample collection to assure there was no blood contamination of the nasal samples. They were considered to be contaminated if any visual indication of blood was noted in the samples either during or after collection. Nasal samples were collected successfully before experimental exposure and 35 days later.

Thirty five days after experimental exposure no statistically significant effect of vaccination or exposure was seen in the nasal class specific anti-PMT titers (Figures 5 through 8). In Figure 5 the nasal level of anti-PMT IgA is shown to be the highest in the experimentally exposed pigs on day 35. Nasal anti-PMT IgG and IgA tended to be higher in the non-vaccinated pigs by day 35 (Figure 6). Figure 7 shows nasal anti-PMT IgG was higher in non-vaccinated and experimentally exposed pigs than in vaccinated and non-experimentally exposed pigs on day 35 ($p=0.08$). The non-vaccinated, not experimentally exposed and the vaccinated, experimentally exposed pigs both had very close to identical increases in anti-PMT IgG levels. The nasal anti-PMT IgA levels of the non-vaccinated pigs tended to increase by day 35 while the level in the pigs that were vaccinated remained steady (Figure 8). This trend appeared to be independent from experimental exposure status.

Figure 5. Effect of exposure to toxigenic *P. multocida* on nasal anti-PMT IgG and IgA (log base 2) in pigs.

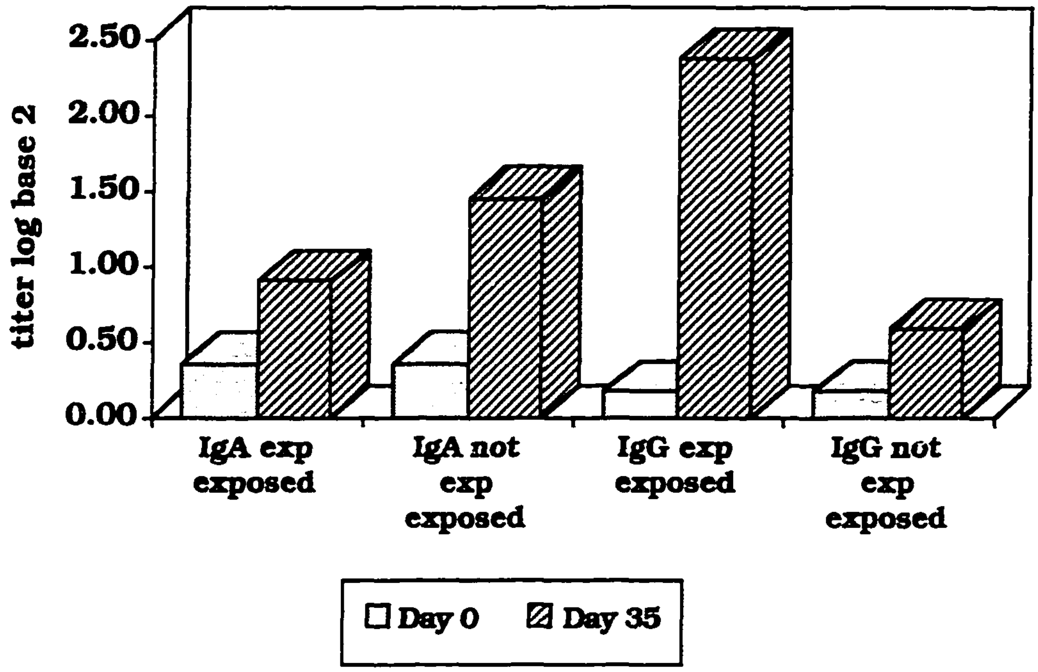


Figure 6. Effect of vaccination with a bacterin/toxoid atrophic rhinitis vaccine on nasal anti-PMT IgG and IgA (log base 2) in pigs.

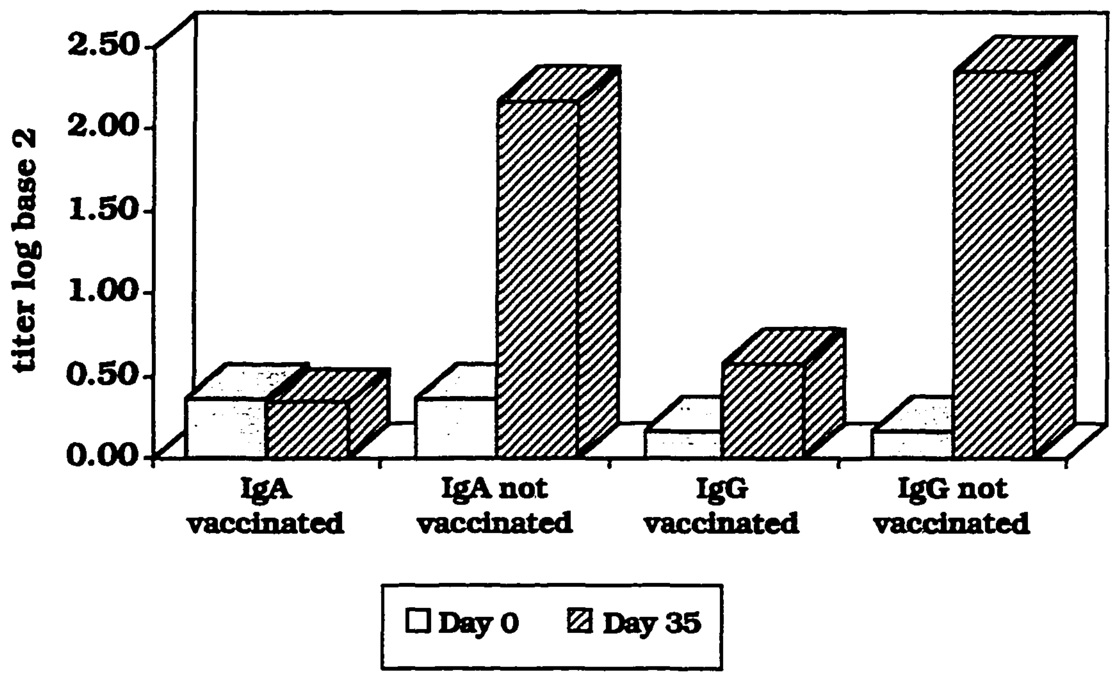


Figure 7. Anti-PMT IgG in nasal secretions by vaccination and experimental exposure treatment groups

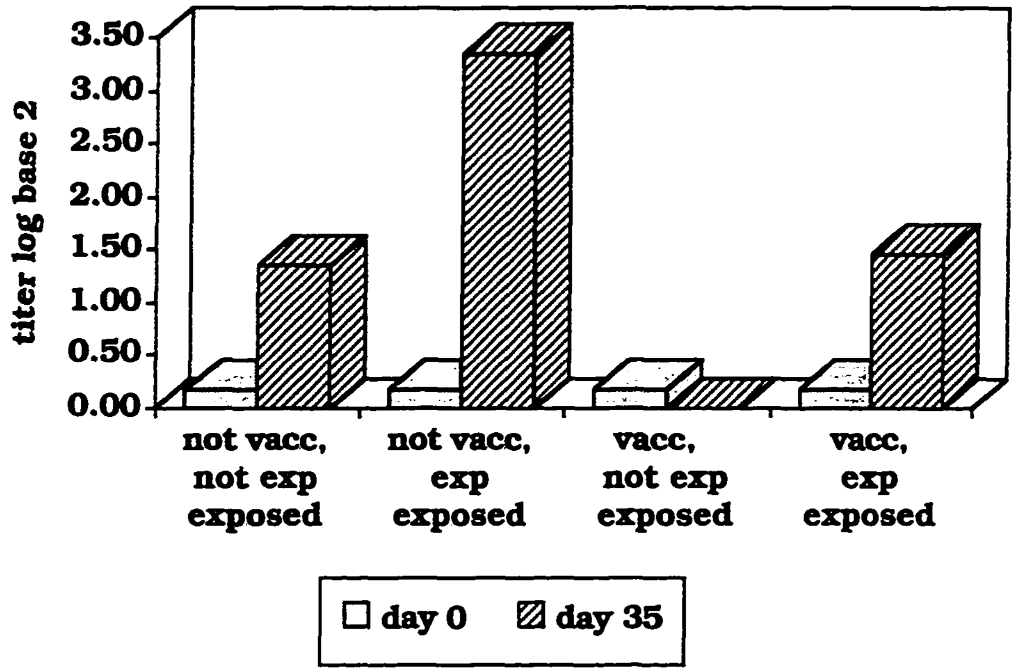
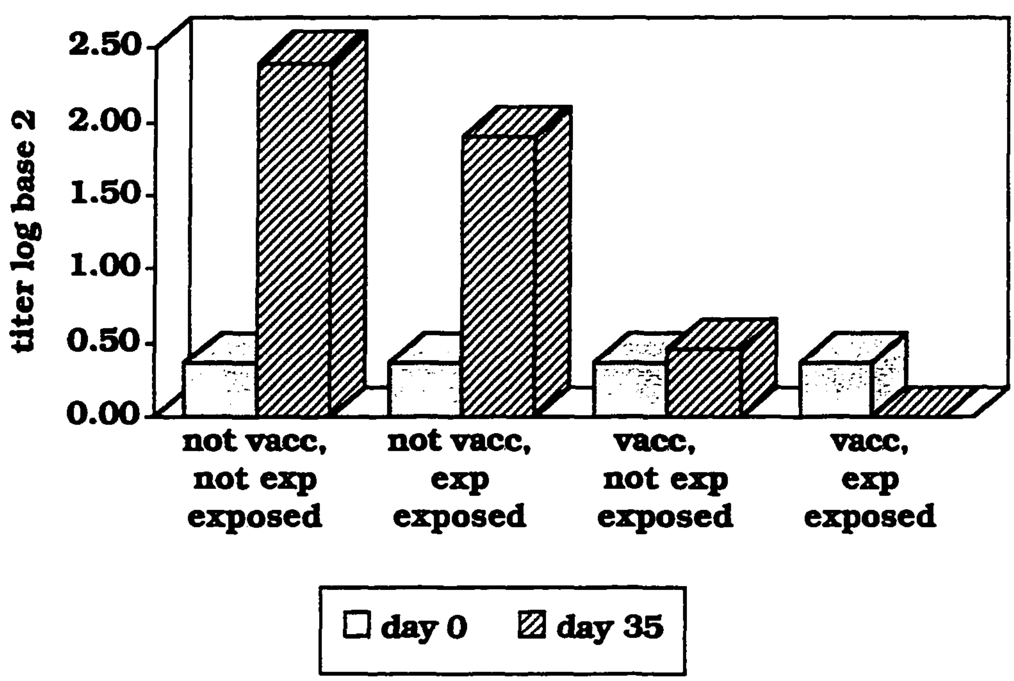


Figure 8. Anti-PMT IgA in nasal secretions by vaccination and experimental exposure treatment groups.



Discussion

Vaccination programs designed to prevent the clinical and economic effects of PAR are widely used in pork production. For many producers the product and labor costs associated with these programs are considered a cost of doing business. The wider acceptance of production technologies such as all in - all out scheduling and segregated early weaning have given the industry the opportunity to refine production systems and examine the cost effectiveness of what was once considered the norm. The economic challenges of today's pork industry do not allow production practices to be followed simply because they are traditional. Each has to be able to demonstrate cost effectiveness on its own merit.

To be able to best assist the pork producer in determining the costs and benefits of a PAR vaccination program, the epidemiology of toxigenic *P. multocida* has to be further defined. One of the key steps in this process is to identify when the organism transfers to susceptible individuals.

In vitro culture has been the traditional method to show the presence of *P. multocida* in an individual. One of the problems with this technique has been an inconsistency in the ability to recover the organism (10,11).

Following upper respiratory infection with *P. multocida*, one of the following scenarios is likely to happen. First, it is possible the pig could mount either a specific immune mediated defense or a nonspecific physical defense that results in the clearing of the organism. Second, protection may be formidable enough to decrease the organism numbers, but not completely eliminate them. Last, the *P. multocida* may be able to establish colonization and multiply. With the first two options, recovery of the organism by nasal swabs will probably be unsuccessful. It is possible that some cyclical combination of proliferation and partial clearing could occur, also affecting the chances of the organism being recovered from carrier animals at any one specific time. These scenarios illustrate why inconsistent recoverability may result in false negative findings, even though in vitro culture has traditionally been used to demonstrate infection.

Table 3 shows that in this study successful recovery of toxigenic *P. multocida* varied by the day of sampling among rooms and among individual pigs. Recovery of the organisms on each sampling day, from even the experimentally exposed pigs, was not consistent. Only 6 of the 10 experimentally exposed pigs yielded recoverable *P. multocida* during the trial.

Table 2 shows two separately housed, experimentally exposed pigs were culture positive on only one day during the trial (number 242, room 8 on day 14 and number 237, room 9 on day 70). None of the cohorts in their isolation rooms were culture positive throughout the trial. This suggests the two culture positive pigs did not clear the organism but had suppressed the numbers enough that they were not recoverable, except for the one day during the trial.

Table 2 also shows transmission to non-experimentally exposed pigs was demonstrated by culture as early as 7 days post experimental exposure (pig 235) with infection continuing as late as day 70 (pig 247). This was expected because the cohort pigs were housed, fed and watered in common.

No vaccinated, non-experimentally exposed pigs were culture positive on any of the sampling dates suggesting protection from transmission by vaccination. *P. multocida* bacterin and toxoid vaccinated, non-experimentally exposed pigs were statistically less likely to have a positive culture on day 7 than were vaccinated and experimentally exposed pigs (Table 3).

Table 4 shows the highest rhinitis scores tended to be in the pigs that were neither vaccinated nor experimentally exposed. The lowest scores tended to be in the pigs that, although they were experimentally exposed, were also vaccinated. These pigs however, were statistically the same as the pigs that were not vaccinated and experimentally exposed (the group that would be expected to have the highest rhinitis scores) so the protective effect of vaccination against gross PAR lesions is questionable.

Anti-PMT IgG in the sera (Figure 1) appears to be a good indicator of exposure. As could be expected with an experimental exposure protocol designed to cause enough nasal mucosal damage to aid colonization, those pigs that were experimentally exposed had apparently absorbed enough PMT to cause a rapid initial rise in antibody. Pigs not experimentally exposed also had an initial increase, but not as fast or to the level of the other pigs, followed by a significant titer rise toward the end of the study.

This serologic profile could support the hypothesis that the non-experimentally exposed pigs became exposed by transmission within the first 21 days. As a group, their anti-PMT IgG titer then leveled, as exposure decreased. Between days 70 and 98 this group appears to be again exposed to toxin, causing a significant rise in titer by day 128. This exposure is most likely due to either recolonization from a second wave of transmission or recrudescence of a lowered level of infection.

Serum anti-PMT IgA does not appear to be a sensitive indicator of exposure (Figure 3). There was a tendency for an initial increase in titer in the experimentally exposed pigs by day 21, somewhat more delayed than the anti-PMT IgG. The experimentally exposed and non-exposed pigs showed very similar trends after day 35.

Although vaccination had no statistical effect on either serum anti-PMT IgA or IgG, there was a some indication of overall higher anti-PMT IgG and lower anti-PMT IgA titers in the vaccinated pigs (Figures 2 and 4).

Because of the efforts to ensure nasal samples were collected without visual contamination by blood, only the collections done pre-experimental exposure and on day 35 could be analyzed. This limits the value of looking at the nasal anti-PMT titers over time but still gives some indication of the movement of the organism within the population.

Measuring the nasal anti-PMT IgG supports the supposition that experimental exposure causes mucosal irritation that could lead to IgG transudation as well as PMT absorption. All pigs received intranasal acetic acid according to the experimental protocol. Figure 5 shows those pigs that were experimentally exposed had the highest nasal anti-PMT IgG on day 35. The high infectious dose of toxigenic P. multocida given to the experimentally exposed pigs may have caused enough inflammation to persist to day 35. Because this is only one day post-experimental exposure however, it is not possible to tell whether this titer is decreasing from a previous higher level or on the rise.

The nasal anti-PMT IgA for both exposure groups tended to move together. The nasal IgA level of the non-experimentally exposed group had a greater movement by day 35 than the nasal IgG level of these pigs. This would support the argument for transmission of the organism before day 35, most probably at a level not great enough to cause extensive mucosal damage and transudation of IgG onto the mucosal surface.

Examining the effect of vaccination on nasal titers (Figure 6) and the interaction of vaccination and exposure (Figures 7 and 8) gives the most striking evidence of transmission and the protective effect of vaccination. In Figure 6 the non-vaccinated pigs show a greater nasal IgA and IgG response than the vaccinated pigs.

This response must be from infection, the source of which is seen in Figures 7 and 8. Those pigs that were not vaccinated and non-experimentally exposed should be the most susceptible to transmission.

The increase in nasal anti-PMT IgA of this group mirrors the increase in the not vaccinated and experimentally exposed pigs. Nasal IgA titers can be used as an indication of transmission.

Likewise, the nasal IgA titers of the vaccinated, non-experimentally and experimentally exposed pigs were together, at a low level, on day 35. This helps support the contention of vaccination protection from transmission seen in Figure 6.

It appears there are different abilities of all of these measurements to detect transmission. Turbinate damage is the least specific indication and does not correspond well with other methods. The environment of these pigs in the research facilities was, subjectively, better than that of pigs in most commercial units. Singling out the environment as a cause of gross lesions does not seen reasonable. The possibility does exist some other infectious agent not accounted for in this study caused the lack of gross lesion similarity between treatment groups.

If absorption of toxin can be assumed to take place, sera anti-PMT IgG appears to be a better indicator of exposure than sera anti-PMT IgA. In the nasal passages, measuring anti-PMT IgA is a good indicator of exposure from transmission. Nasal IgG titers were too dependent on a deeper, more inflammatory infection causing transudation to be a reliable indication of transmission.

Culture results and serological and mucosal assay do support each other. The exposed pigs were more likely to be culture positive on day 7 and to have a high anti-PMT IgG titer by that day. Antibody assays and culture results suggested transmission had occurred, especially in non-vaccinated pigs.

Bibliography

1. Ackerman, M.R., R.B. Rimler, J.R. Thurston. 1991. Experimental model of atrophic rhinitis in gnotobiotic pigs. Infection and Immunity. **59**: 3626-3629.

2. Brandtzaeg, P. 1984. Immune functions of human nasal mucosa and tonsils in health and disease. pp 28-95. **Immunology of the lung and upper respiratory tract**. J. Bienenstock, ed. McGraw-Hill Book Co., New York.

3. Chanter, N. and J.M. Rutter. Atrophic rhinitis. pp 162-195. **Pasteurella and Pasteurellosis.** C. Adam and J.M. Rutter, eds. Academic Press, London.

4. Chanter, N. and J.M. Rutter. 1990. Colonization of *Pasteurella multocida* in atrophic rhinitis of pigs and immunity to the osteolytic toxin. Vet Micro. **25**: 253-265.

5. Chanter, N., J.M. Rutter, and A. Mackenzie. 1986. Partial purification of an osteolytic toxin from *Pasteurella multocida.* J Gen Micro. **132**: 1089-1097.

6. Dominick, M.A. and R.B. Rimler. 1986. Turbinate atrophy in gnotobiotic pigs intranasally inoculated with protein toxin isolated from type D *Pasteurella multocida* . Am J Vet Res. **47**: 1532-1536.

7. Foged, N.T., J.P. Nielsen, and K. Barfod. 1990. The use of ELISA determination of *Pasteurella multocida* toxin antibodies in the control of progressive atrophic rhinitis. Proceedings 11th Int Pig Vet Soc Congr, Lausanne. p 49.

8. Foged, N.T., K.B. Pedersen, and F. Elling. 1987. Characterization and biological effects of the *Pasteurella multocida* toxin. FEMS Micro Letters. **43**: 45-51.

9. Frymus, T., E. Müller, B. Franz, K. Petzoldt. 1989. Protection by toxoid-induced antibody of gnotobiotic piglets challenged with the dermonecrotic toxin of *Pasteurella multocida* . J Am Vet Med Assoc. **36**: 674-680.

10. Hoffman, L.J. 1989. Recovery of toxigenic *Pasteurella multocida* and *Bordetella bronchiseptica* from Iowa swine herds affected with atrophic rhinitis. **Iowa State University 1989 Swine Research Report**. ASL-R660, paper ASL-R659. pp 66-67.

11. Hoffman, L.J. 1990. Recoverability of *Pasteurella multocida* from nasal cavities of challenged pigs. **Iowa State University 1990 Swine Research Report**. ASL-R773, paper ASL-R773. pp 63-64.

12. Hoffman, L.J. 1993. Personal communication. Iowa State University. Ames, Iowa.

13. Kimman, T.G., R.A. Brouwers, J.J. Daus, J.T. vanOirschot, and D. vanZaane. 1992. Measurement of isotype-specific antibody responses to Aujezky's disease virus in sera and mucosal secretions in pigs. Vet Immun and Immunopath. **31**: 95-113.

14. Martineau-Doizé. B., J. Menard, C. Girard, J.C. Frantz, and G.P. Martineau. 1991. Effects of purified *Pasteurella multocida* dermonecrotic toxin on the nasal ventral turbinates of fattening pigs: histological observations. Can J Vet Res. **55**: 377-379.

15. Morgan, K.L. and F.J. Bourne. 1980. Immunoglobulin levels in porcine nasal and tracheal secretions - the influence of the method of collection. J of Immun Meth. **37**: 165-173.

16. Morgan, K.L. and F.J. Bourne. 1981. Immunoglobulin content of the respiratory tract secretions of piglets from birth to 10 weeks old. Res in Vet Sci. **31**: 40-42.

17. Rutter, J.M. and A. Mackenzie. 1984. Pathogenesis of atrophic rhinitis in pigs: a new perspective. Vet Rec. **114**: 89-90.

18. Williams, P., M. Hall, and R. Rimler. 1990. Host response to *Pasteurella multocida* turbinate atrophy toxin in swine. Can J Vet Res. **54**: 157-163.

V. USE OF RESTRICTION ENDONUCLEASE ANALYSIS TO STUDY TOXIGENIC *PASTEURELLA MULTOCIDA* TRANSMISSION IN SWINE

A paper to be submitted to the journal Clinical Microbiology

Paul L. Sundberg, Mark A. Wilson,
Eldon K. Uhlenhopp and Lorraine J. Hoffman

Abstract

Restriction endonuclease analysis (REA), serotyping, and toxigenicity were used to follow transmission of a toxigenic *Pasteurella multocida* strain P4148, among commingled feeder pigs. Twenty pigs (14 to 18 kg) were nasal swabbed. Swabs were cultured, and cultures were evaluated for *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* status; bacterial cultures from nasal swabs of all 20 pigs were negative for these pathogens. Pigs were randomly divided into two groups, both groups received an intranasal inoculation of a sterile toxigenic *B. bronchiseptica* sonicate; one group (designated as exposed) was inoculated with a toxigenic *P. multocida* 48 hours later. The remaining group (designated as nonexposed) was not inoculated with a toxigenic *P. multocida*. Characterization of isolates from nasal swab cultures demonstrated *P. multocida* transmission from the exposed to nonexposed pigs. Transmission of toxigenic *P. multocida* strain P4148 was confirmed by REA, serotyping, and *P. multocida* toxin ELISA. Background nontoxigenic *P. multocida* strains were recovered from the pigs and these strains were the same somatic type as strain P4148. The background nontoxigenic strains were easily differentiated from strain

P4148 by REA and toxigenicity. REA confirmed that the profile of each toxigenic isolate recovered from a nasal swab was identical to the profile of P4148. Other toxigenic *P. multocida* were not isolated or identified from any of the 20 pigs. REA is a useful epidemiological tool that, when used with serotyping and toxigenicity assay, can further differentiate *P. multocida* strains during clinical trials.

Introduction

Swine progressive atrophic rhinitis (PAR) is a specific disease entity caused by infection with toxigenic *Pasteurella multocida* (18). The clinical syndrome associated with PAR is of considerable economic importance to the swine industry (4, 9, 15). Post mortem examinations conducted at slaughter plants have shown that up to two-thirds of the marketable age swine in the United States show signs of turbinate atrophy, one of the overt manifestations of PAR (15).

The etiologic agent of PAR is toxigenic *P. multocida*. Colonization and toxigenicity are the most important *P. multocida* virulence determinants (14). Because nontoxigenic strains of *P. multocida* are normal residents on the nasal mucosa of healthy pigs (16), the determination of toxigenicity is central in reaching a PAR diagnosis. Animal models (6), cytotoxicity assays (6), colony blot immunoassays (20), ELISA techniques (8) and DNA probes (19) have been used to detect the presence of or potential for *P. multocida* toxin in cultures.

Capsular and somatic serotyping, phage typing and restriction endonuclease assay (REA) have been used together with toxigenicity assays to study the epidemiology of PAR and determine a likely period of toxigenic *P. multocida* exposure. Once thought to be transmitted principally from sow to piglets during the first weeks of life, there is increasing evidence toxigenic *P. multocida* initially infects a few nursing pigs which become carriers. Primary herd transmission then occurs among cohort pigs post weaning (17, 23).

Recently REA has been used to allow greater differentiation of *P. multocida* strains than that afforded by phenotyping techniques (10, 22, 23, 24). Cross-sectional observational studies support the use of REA in combination with classical capsular and somatic typing as an epidemiologic tool to provide information regarding *P. multocida* identification. Isolates of identical capsular and somatic types can be further classified by REA pattern.

REA has furthered the epidemiological study of pneumonic pasteurellosis, however, only one study has addressed the relationship among PAR, capsular and somatic type, toxigenicity and REA (10). Data from that study suggest one REA pattern can come from identical capsular type strains that differed in toxigenicity based on the guinea pig dermonecrosis assay.

Transmission studies in field and experimental situations have demonstrated an inconsistency in the ability to sequentially and quantitatively isolate toxigenic *P. multocida* when the identifying criteria are capsular type and toxigenicity (2, 5, 12, 13). It could not be determined if this inability to consistently recover the organism was due to clearance followed by reinfection, persistent infection with numbers too low to recover, errors in sampling and culture technique or a change in the bacteria, stopping toxin production and thus detection.

The objective of this experiment was to follow transmission, by REA and phenotypic characteristics, of an inoculated toxigenic *P. multocida* among cohort pigs in a clinical trial. REA, serotyping and an ELISA designed to detect presence of toxin were used to determine whether toxigenic *P. multocida* can escape detection upon isolation and subculture.

Materials and Methods

Toxigenic *B. bronchiseptica* (strain P-4609) and toxigenic *P. multocida* (Serotype D:3, strain P4148 - referred to as the reference strain) were supplied by the USDA National Animal Disease Laboratory, Ames, Iowa. Toxigenicity of the *P. multocida* was confirmed by ELISA prior to beginning the project.

Twenty pigs ranging in weight from 14 to 18 kg were randomly assigned, five pigs to each of four rooms. The rooms were designed for isolation with separate feeders, water outlets, ventilation and waste disposal. All pigs were clinically normal and negative for *B. bronchiseptica* and toxigenic *P. multocida* by nasal swab culture. They were fed, ad libitum, a corn-soybean based diet without antibiotics.

The pigs in each room, after 14 day acclimation, were randomly divided into exposed and nonexposed groups. Two pigs in two rooms and three pigs in each of the remaining rooms were exposed. The other pigs in all rooms were not exposed.

All pigs were held in an upright position and inoculated via flexible, polyethylene catheter attached to a 3 ml. syringe. A sterile sonicate of a toxigenic *B. bronchiseptica* was instilled in the nasal passages of all pigs. Forty-eight hours later, 1 ml. of a live culture suspension of toxigenic *P. multocida* , diluted in phosphate buffered saline to a density equivalent to that of a McFarland 1 nephelometer standard, was instilled in each nasal passage of those pigs assigned to the exposed group. Pigs in the nonexposed group received sterile culture media, the same as that used to grow the *Pasteurella multocida*. Sonicates and cultures were prepared according to the protocol of Ackermann, Rimler and Thurston (1).

After wiping the external surface of the nose with an individual gauze sponge, a cotton-tipped miniswab[a] was used to sample the conchae of each nostril. The transport media vial with each swab was not broken following collection as the swabs were submitted for culture immediately after sample collection. Samples were collected for culture and analysis immediately preinoculation and on days 3, 7, 14, 21 and 28 postinoculation.

Each swab was immersed in 2.0 ml of sterile water and mixed by vortex mixer. On each of a 5% sheep blood agar plate and a plate containing a selective medium using a Columbia blood agar base with neomycin and bacitracin (12, 13) 200 µl were then placed. Each plate

---

[a] Mini-Tip Culturette®, Becton Dickinson Microbiology Systems, Cockeysville, Maryland

was streaked for isolation.

After 48 hours of aerobic incubation at 37°C, up to five isolated colonies visually identified as possible *P. multocida* on each plate were given coded identification. Each colony was then transferred to a 5% sheep blood agar plate and also into dextrose, sucrose, maltose, mannitol, lactose, indole and urea media. Those strains which produced reactions typical of *P. multocida* were then transferred from the 5% sheep blood agar plate to a BHI slant for REA. The remaining colonies on the 5% sheep blood agar plate were used for ELISA determination of toxin production.

Toxigenicity determination, serotyping and REA

Toxin production was detected using a commercially available ELISA kit[b] as directed by the manufacturer. Each assay was done in duplicate.

The coded BHI slants of *P. multocida* were submitted for capsular and somatic typing and REA. Associations of isolates could not be determined without knowledge of the coding system. Capsular typing was performed by the hyaluronidase inhibition assay of Carter (3). Somatic antigen type was determined by the method of Heddleston, et al. (11) Chromosomal DNA isolation, restriction endonuclease digestion using *HhaI*[c], electrophoresis and photography were performed by the methods of Wilson, et al. (22)

Statistical analysis was performed using the Chi squared test.

---

[b] DAKO PMT ELISA®, DAKO Corporation, Carpinteria, California

[c] Life Technologies, Inc., Gaithersburg, Maryland

Results

Culture and toxigenicity

Table 1 details the results of the culture and toxigenicity assay. Toxigenic *P. multocida* was isolated from nasal swabs of exposed and nonexposed pigs as early as three days post exposure. Recovery of a toxigenic isolate occurred from exposed pigs in rooms 1, 2 and 4. Toxigenic *P. multocida* was recovered from at least one nonexposed pig in rooms 1 and 4. No toxigenic *P. multocida* was recovered from exposed or nonexposed pigs in room 3. Consistent recovery of *P. multocida* was shown in only one pig, pig number 2, which was exposed and housed in room 1.

Recoverability of toxigenic *P. multocida* varied greatly (Table 2). Of 60 exposed pig samples, 15 had a positive reaction for *P. multocida* toxin after culture. Recoverability from exposed pigs varied between rooms (Table 2) and, over time, within rooms (Table 1).

Toxigenic *P. multocida* was recovered from exposed and nonexposed pigs. Table 3 identifies the pigs from which a *P. multocida* toxin positive culture was grown during the course at the project. Rooms 1 and 4 each contained 1 nonexposed pig from which a toxin positive culture was obtained. There was no significant difference between the number of exposed pigs and the number of nonexposed pigs yielding *P. multocida* toxin positive cultures (Table 3).

TABLE 1. Culture isolation and toxigenicity results.

Pig ID	Exposure	Room	Culture, by day: Pre-Exp	3	7	14	21	28	35
1	Not[a]	1	-[c], B_1[d]	-, B_1	-, B_1, B_2[f]	-, B_1	-	-	-, B_1
2	Exp[b]	1	-	+[e]	+, B_1, B_2	+	+	+	+
3	Exp	1	-	+	+	-	+	+	+
4	Not	1	-	-	+	+	-	-	+
5	Exp	1	-	+	-	-	-	-	-
6	Not	2	-	-	-	-	-	-	-
7	Exp	2	-	-	-	-	-	-	-
8	Not	2	-	-	-	-	-	-	-
9	Exp	2	-	-	-	-	-	-	-
10	Exp	2	-	-	+	-	-	-	-
11	Not	3	-	-	-	-	-	-	-
12	Not	3	-	-	-	-	-	-	-
13	Exp	3	-	-	-	-	-	-	-
14	Exp	3	-	-	-	-	-	-	-
15	Not	3	-	-	-	-	-	-	-
16	Not	4	-	-	-	-	-	-	-
17	Not	4	-	+	+	-	-	+	+
18	Not	4	-	-	-	-	-	-	-
19	Exp	4	-	-	-	-	-	-	-
20	Exp	4	-	-	+	+	-	-	-

[a] Not exposed
[b] Exposed
[c] Culture *P. multocida* toxin negative
[d] Background REA type 1, nontoxigenic *P. multocida*
[e] Culture *P. multocida* toxin positive
[f] Background REA type 2, nontoxigenic *P. multocida*

TABLE 2. Number of nasal swab primary cultures positive for *P. multocida* toxin.

Room	Exposed Pigs	Not Exposed Pigs
1	12 / 18[a]	3 / 12[a]
2	1 / 18	0 / 12
3	0 / 12	0 / 18
4	2 / 12	4 / 18
Total	15 / 60[b]	7 / 60[b]

Values with same superscripts are significantly different, $p < 0.1$

TABLE 3. Number of pigs yielding culture of *P. multocida* which produced toxin

Room	Exposed Pigs	Not Exposed Pigs
1	3 / 3[a]	1 / 2[a]
2	1 / 3	0 / 2
3	0 / 2	0 / 3
4	1 / 2	1 / 3
Total	5 / 10[b]	4 / 10[b]

Values with same superscripts are significantly different, $p < 0.1$

Restriction endonuclease analysis and phenotypic typing

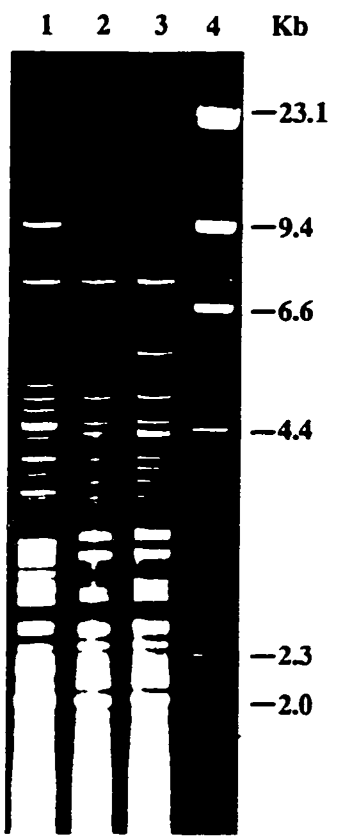
Eighty-six colonies from 27 nasal swab cultures were identified as *P. multocida* by visual and biochemical criteria. All were negative for capsular type A using Carter's hyaluronidase capsular typing test. Eighty-four of the 86 colonies were found to belong to somatic type 3 when referenced by the Heddleston somatic typing assay. Two colonies were somatically untypable. The *P. multocida* reference strain was also somatic type 3 and negative to capsular type A.

Fifty-one of the 86 colonies were toxigenic and found to have an REA pattern identical to the inoculated reference strain P4148 (Figure 1, lane 1). Included in these 51 colonies were isolates from each of the two nonexposed pigs that yielded toxin positive cultures.

Thirty-three of the 35 colonies were nontoxigenic and possessed the REA pattern shown in Figure 1, lane 2 (*P. multocida* background type 1). The remaining two colonies were nontoxigenic and found to have the REA pattern of Figure 1, lane 3 (*P. multocida* background type 2). All 35 colonies with REA patterns differing from the reference strain were isolates from two pigs, numbers 1 and 2, in room 1.

Background *P. multocida* (profile type 1) was isolated from pig numbers 1 and 2 before the date of exposure. The identical isolate was recovered from pig 1 on days 3, 7, 14 and 35 and from pig 2 on day 7 (Table 1). Background *P. multocida* (profile type 2) was isolated from pig numbers 1 and 2 on day 7 (Table 1). No other nontoxigenic *P. multocida* was recovered.

FIG 1. Agarose gel DNA fingerprint profiles of representative *P. multocida* colonies. Lanes: 1, *HhaI* profile of the inoculated toxigenic *P. multocida* reference strain and all recovered toxigenic isolates; 2, *HhaI* profile of the nontoxigenic background type 1 *P. multocida*; 3, *HhaI* profile of the nontoxigenic background type 2 *P. multocida*; 4, DNA from lambda phage digested with *Hind* III.



Discussion

REA has been used in observational cross section studies in which transmission has been suggested (10, 22, 23, 24). In this project, toxigenic *P. multocida* transmission was implied by phenotypic characteristics (capsular and somatic typing and toxigenicity) and confirmed by REA.

During initial epidemiological studies of *P. multocida*, transmission was defined by recovery of a capsular and somatic type *P. multocida* that was the same as the inoculated strain. The discovery of the *P. multocida* toxin gave an additional tool with which to differentiate strains. It is possible for separate isolates to have identical capsular and somatic types but different toxigenic capabilities. REA gives additional support to epidemiological studies by further differentiating isolates which are similar in other ways.

Table 1 shows the inconsistent recoverability of *P. multocida* from pigs exposed to the organism. This was not surprising and agrees with the results of previous studies (1, 3, 5, 11).

A recovery pattern such as this is of concern when sampling pigs in a clinical setting or verifying toxigenic *P. multocida* free status prior to a clinical study. Using only phenotypic characteristics to detect transmission may lead to a false sense of security. Defining a positive result as detection of *P. multocida* toxin, three exposed pigs were culture negative on day three, then positive on day seven. Pig number 17 would have been considered negative had initial samples been taken on day 14 or day 21 (Table 1). Had this pig then been assigned to a toxigenic *P. multocida* free group used to monitor transmission, transmission would have mistakenly appeared to have occurred when the pig cultured positive on day 28. Using REA, the toxigenic isolates recovered during this experiment, although recovered on an intermittent basis, were confirmed to be identical to that of the inoculated reference strain. Determination of toxigenicity alone could not have differentiated a background toxigenic strain, that was initially not detected, from the inoculated toxigenic strain.

Multiple colonies from the primary nasal swab cultures were used to decrease the likelihood of not sampling genotypically unique colonies that may be visually identical. Each group of up to five subcultured colonies coming from a primary nasal culture was found to have identical capsular and somatic types, toxigenicity and REA fingerprint pattern.

Previous studies have focused on pneumonic pasteurellosis without correlating toxigenicity to REA pattern (23, 24). Harel, et al., reported identical DNA fingerprints for toxin positive and toxin negative isolates (10). The toxA gene, encoding for toxin production, has been identified as chromosomal (19). As such, a DNA fingerprint of a toxigenic isolate may differ from that of a nontoxigenic isolate, if the lack of toxigenicity is due to toxA deletion. Differentiation between toxigenic and nontoxigenic *P. multocida* strains by REA was shown in this study. Using *HhaI*, the nontoxigenic background *P. multocida* strains were easily distinguishable from the inoculated toxigenic reference strain.

Toxigenicity determination method and restriction endonuclease selection may affect results. *HhaI* digestion results in distinct fingerprint profiles easily distinguishable with the naked eye. Additionally, this study used ELISA for toxin detection rather than a less sensitive (7), less reproducible and more subjective (21) bioassay.

The only toxigenic strain isolated was the inoculated reference strain. It produced a unique DNA fingerprint pattern distinguishable from the recovered nontoxigenic background strains. To determine a range and comparison of toxigenic and nontoxigenic *P. multocida* REA fingerprints surveying herds affected with clinical progressive atrophic rhinitis has yet to be done.

Bibliography

1. Ackermann, M. R., R. B. Rimler, and J. R. Thurston. 1991. Experimental model of atrophic rhinitis in gnotobiotic pigs. Infect. and Immun. **59**: 3626-3629.

2. Ackermann, M. R., N. F. Cheville, and J. E. Gallagher. 1991. Colonization of the pharyngeal tonsil and respiratory tract of the gnotobiotic pig by a toxigenic strain of *Pasteurella multocida* type D. Vet. Pathol. **28**: 267-274.

3. Carter, G. R., and S. N. Rundell. 1975. Identification of type A strains of *Pasteurella multocida* using a staphylococcal hyaluronidase. Vet. Rec. **93**: 343.

4. Chanter, N., and J. M. Rutter. 1989. Atrophic Rhinitis. In C. Adam and J. M. Rutter (ed.), Pasteurella and Pasteurellosis. Academic Press, London.

5. Chung, W., L. R. Bäckstrom, T. Conrad, and M. T. Collins. 1990. A comparison of different challenge methods for induction of atrophic rhinitis in pigs. APMIS 1990. **98**: 442-452.

6. deJong, M. F., H. L. Oei, and G. J. Tetenburg. 1980. AR-pathogenicity tests for *Pasteurella multocida* isolates. p 211. Proc. 6th IPVS Congr., Copenhagen 1980.

7. Foged, N. T. 1988. Quantification and purification of the *Pasteurella multocida* toxin by using monoclonal antibodies. Infect. and Immun. **56**: 1901-1906.

8. Foged, N. T., J. P. Nielsen, and K. B. Pedersen. 1988. Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by enzyme-linked immunosorbant assay. J. of Clin. Micro. **26**: 1419-1420.

9. Frymus, T., E. Mü, B. Franz, and K. Petzoldt. 1989. Protection by toxin-induced antibody of gnotobiotic piglets challenged with the dermonecrotic toxin of *Pasteurella multocida*. J. Vet. Med. **36**: 674-680.

10. Harel, J., S. Côté and M. Jacques. 1990. Restriction endonuclease analysis of porcine *Pasteurella multocida* isolates in Quebec. Can. J. Vet. Res. **54**: 422-426.

11. Heddleston, K. L., J. E. Gallagher, and P. A. Rebers. 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Dis. **16**: 925-936.

12. Hoffman, L. J. 1989. Recovery of toxigenic *Pasteurella multocida* and *Bordetella bronchiseptica* from Iowa swine herds affected with atrophic rhinitis. pp 66-67. Iowa State University 1989 Swine Research Report. ASL-R660.

13. Hoffman, L. J. 1990. Recoverability of *Pasteurella multocida* from nasal cavities of challenged pigs. pp 63-64. Iowa State University 1990 Swine Research Report. ASL-R773.

14. Letellier, A., D. Dubreuil, G. Ray, J. M. Fairbrother and M. Jacques. 1991. Determination of affinity of *Pasteurella multocida* isolates for porcine respiratory tract mucus, and partial characterization of receptors. Am. J. Vet. Res. **52**: 34-39.

15. Muller, R. D., and P. B. Abbott. 1986. Estimating the cost of respiratory disease in hogs. Anim. Health Nutr. **41**: 30-35.

16. Nakai, T., K. Kume, H. Yoshikawa, T. Oyamada, and T. Yoshikawa. 1988. Adherence of *Pasteurella multocida* or *Bordetella bronchiseptica* to the nasal epithelial cell *in vitro*. Infect. Immun. **56**: 234-240.

17. Nielsen, J. P., P. Kolkman, and S. Rosendal. 1992. Infection with toxigenic *Pasteurella multocida* and growth rate in a cohort of pigs. Proc. 12th IPVS Congr., The Hague 1992.

18. Pedersen, K. B., J. P. Nielsen, N. T. Foged, F. Elling, N. C. Nielsen, and P. Willeberg. 1988. Atrophic rhinitis in pigs: proposal for a revised definition. Vet. Rec. **122:** 190-191.

19. Petersen, S. K., and N. T. Foged. 1989. Cloning and expression of the *Pasteurella multocida* toxin gene toxA, in Escherichia coli. Infect. and Immun. **57:** 3907-3913.

20. Rutter, J. M., and P. D. Luther. 1984. Cell culture assay for toxigenic *Pasteurella multocida* from atrophic rhinitis of pigs. Vet. Rec. **114:** 393-396.

21. Vena, M. M., B. Blanchard, D. Thomas, and M. Kobisch. 1991. Adherence of *Pasteurella multocida* isolated from pigs and relationships with capsular type and dermonecrotic toxin production. Ann. Rech. Vet. **22:** 211-218.

22. Wilson, M. A., R. B. Rimler, and L. J. Hoffman. 1992. Comparison of DNA fingerprints and somatic serotypes of serogroup B and E *Pasteurella multocida* isolates. J. of Clin. Micro. **30:** 1518-1524.

23. Zhao, G., C. Pijoan, and M. P. Murtaugh. 1992. Epidemiology of *Pasteurella multocida* in a farrow-to-finish swine herd. Can. J. Vet. Res. **57:** 136-138.

24. Zhao, G., C. Pijoan, M. P. Murtaugh, and T. W. Molitar. 1992. Use of restriction endonuclease analysis and ribotyping to study epidemiology of *Pasteurella multocida* in closed swine herds. Infect. and Immun. **60:** 1401-1405.

VI. GENERAL CONCLUSION

As the pork production industry has evolved, animal production has become more concentrated. The desire to decrease labor and land appropriated to production while maximizing efficiency has led to greater populations of pigs being housed in spaces small enough to not limit growth and production. This increase in animal concentration also has created an increase in opportunity for transmission of diseases.

Economically preventing diseases from affecting their animal's production has been a goal of farmers since they first started to domesticate animals. An early way to prevent disease was to remove the diseased animals and decrease the concentration of the herd. Modern pork producers may not have this option. Instead, they may need to rely on decreasing exposure, decreasing infection, or stimulating immunity.

The industry is starting to learn how to reduce exposure without decreasing herd concentration. Advances in environmental control have helped decrease exposure by reducing stresses that contribute to shedding and by reducing time of contact between pathogens and animals. Management techniques to reduce horizontal or vertical transmission are becoming industry standards.

One method for the pork producer to reduce transmission among pigs is to increase the level of immunity. To do this, pork producers routinely use vaccination as a management technique. It is generally held that for most situations the higher the antibody titer the greater the protection against transmission and the effects of the pathogen. Any cost-effective improvement in the vaccination process that would result in increased immunity would be adopted by the pork production industry.

In these experiments, beta-hydroxy beta-methyl butyrate (HMB) was used in an attempt to increase the immune reaction to vaccination. Some dose response effect of HMB on antibody production was seen. Supplementation with two grams of HMB once daily caused a response to vaccination that tended to be higher than that in either those given ten grams once daily or controls not receiving HMB. This and the HMB effect on class specific antibody production were variable enough to warrant further study to better define how HMB could be used to mediate an immune response.

For other management techniques to be most useful in decreasing transmission, the epidemiology of the particular organism of interest must be understood. Basic to this understanding is a method to reliably detect transmission.

One classic technique of inferring transmission is by showing seroconversion, a biological indication of antigen exposure to a competent immune system. For it to be an effective indicator, the organism must be in contact with systemic immunocytes that are capable of producing an antibody response. There must also be enough time for the immunocytes to produce antibodies in detectable quantities.

For bacterial respiratory infections, the first exposure to the organism comes from contact with the body's mucosal surfaces. With toxigenic *P. multocida* and the upper respiratory tract, the opportunity for toxin exposure to both the mucosal and systemic immune systems happens after absorption of the toxin. It is not unreasonable to expect antibody production by both systems. The nature of the reaction, the period within which it occurs and its amount could show exposure from transmission.

In these studies, this was the case. Serum anti-PMT IgG was an effective indicator of transmission by day 21 after mingling with experimentally exposed pigs. A second wave of exposure between days 70 and 98 was also detected serologically.

Anti-PMT IgA on the nasal mucosal surface also could be used to show transmission. Because of constraints from collection, the earliest sample for assay was from day 35 post-mixing with experimentally exposed pigs. This leaves open the question of how early during an infection mucosal IgA could be used to show transmission, but it does show the ability of assay for class specific antibody from the mucosal surface to indicate exposure. Further experimentation is needed to define the time needed for detectable antibody production on the nasal surface.

Another method of showing transmission is by recovery of the organism. With *P. multocida*, recovery from even experimentally exposed individuals is unreliable. It is not known whether this is a factor of the sampling technique, at least partial clearance of the organism with reinfection or recrudescence, or recovery of different *P. multocida* organisms with similar phenotypic characteristics. The inconsistent recovery brings into question the reliability of this technique for diagnosis, especially on an individual animal basis. Even when using this on a herd basis, one has to be careful not to underestimate prevalence based on one sampling.

With experimentally inoculated animals, the same problems with unreliable recovery exist. Because of this, as with recovery from naturally exposed animals, using culture for detection of transmission should be equally suspect. Chance recovery of a previously undetected strain of bacteria that is phenotypically identical to an inoculated strain would interfere with data interpretation and lead to false conclusions about transmission and prevalence. Restriction endonuclease analysis of the bacterial DNA was used to show that, at least under the conditions of this experiment, recovery of the organism was a positive indication of the transmission of the inoculated strain.

Finally, while helping to answer some questions about the epidemiology of toxigenic type D *P. multocida*, this research also demonstrated a technique that could have longer reaching effects. Whether bacterial or viral, a pig first contacts most pathogens on a mucosal surface. This gives the mucosal surface the first opportunity to preclude infection and prevent its economic effects. This is why it is so important to understand pathogen interactions with this immune system.

Sampling mucosal surfaces directly, without dilution or contamination from washing and flushing gives the opportunity to detect exposure and immune reaction in a very site specific manner. This technique could be applied to study the epidemiology of many pathogens. Salmonella may be contacted through the nasal passages as a pig sniffs and roots in a contaminated environment. Perhaps by similar sample collection and ELISA use, a greater understanding of salmonella exposure and immune interaction could be gained. Systems of salmonella control and elimination that could be helpful in food safety initiatives could then be developed.

Viral infections such as Porcine Respiratory and Reproductive Syndrome (PRRS) are greatly affecting the pork production industry. One possible portal of entry into the body and site of viral replication is the nasal mucosa. Using similar methods, perhaps we could gain a better understanding of the role mucosal exposure and immune reaction plays in the pathogenesis of this syndrome.

Whatever pathogen is studied, if it causes a measurable effect on the profitability of the pork producer and it is transmitted by contact with a mucosal surface, these techniques may be helpful in defining its epidemiology. Further study should help refine their reliability and usefulness.

APPENDIX

Table 1. Sera HMB levels (micromolar) in gilts fed HMB top-dressed on feed once daily (n = 12 for each HMB treatment).

	Grams of HMB fed per day		
Days	0	2	10
-38	3.5	3.3	4.7
-28	3.3	45.3	149.5
-14	4.8	30.8	136.9
-7	3.4	42.4	131.1
1	4.1	65.4	105.6
7	7.4	24.8	149.3
14	4.6	21.8	140.4
21	5.2	17.7	102.1

Ave SEM=15; all values are significantly different across treatments at $p<0.01$

Table 2. Anti-tetanus sera titers (log base 2) of gilts fed varying levels of HMB once daily (titer (n)).

	Grams of HMB fed per day		
Day	0	2	10
-38	7.5 (10)	7.5 (11)	7.5 (11)
-28	11.6 (10)	13.0^{A} (10)	10.8^{A} (11)
-14	11.9^{A} (7)	$14.4^{A,B}$ (7)	10.8^{B} (9)
-7	11.1 (10)	11.9 (10)	10.8 (10)
1	10.3^{A} (10)	11.6^{A} (10)	11.3 (8)
7	9.7 (10)	10.9 (11)	10.3 (9)
14	9.0 (10)	9.9 (11)	10.0 (8)
21	$10.9^{A,B}$ (6)	8.3^{B} (7)	7.6^{A} (5)

Ave SEM=0.81 ; For like letters across rows, $p<0.10$.

Table 3. Anti-pseudorabies sera titers (log base 2) of gilts fed varying levels of HMB once daily (titer (n)).

	Grams of HMB fed per day		
Days	0	2	10
-38	1.64 (12)	1.64 (12)	1.64 (12)
-28	2.50^{A} (4)	3.34^{A} (3)	2.57 (4)
-14	3.91 (12)	3.35 (12)	2.99 (12)
-7	2.92^{B} (4)	3.65^{B} (5)	2.95 (6)
1	1.94 (8)	2.24^{C} (7)	1.45^{C} (7)
7	2.92 (12)	3.29 (11)	3.08 (11)
14	2.49 (11)	2.80 (10)	2.62 (9)
21	2.60 (11)	2.29 (11)	2.32 (9)

Ave SEM = 0.5; For like letters across rows, $p<0.10$.

Table 4. Sera IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 1 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	1.58^{C} (5)	0.70^{A} (6)	0.23^{B} (2)	0.84^{C}
	no	$4.79^{A,B,C}$ (1)	2.48 (1)	1.96^{D} (1)	3.08^{C}
	MEAN	3.18	1.59	1.09	

Ave SEM=1.1; $^{A,B}p<0.05$, $^{C,D}p<0.1$

Table 5. Sera IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 7 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	2.69	0.94	2.48	2.04
	no	1.03	0.70	0.12	0.62
	MEAN	1.86	0.82	1.30	

Ave SEM=1.39

Table 6. Sera IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 14 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	$2.45\ (3)^{A}$	$-0.64\ (3)^{A,B}$	$1.83\ (3)^{B}$	1.21
	no	-0.58 (1)	-0.19 (1)	na	-0.38
	MEAN	0.93	-0.41	1.83	

Ave SEM=0.95; For like letters, $p<0.10$

Table 7. Sera IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 21 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.42^{D} (3)	0.39^{E} (5)	0.40^{F} (2)	0.40^{C}
	no	$3.85^{D,E,F,G}$(1)	na	-0.10^{G} (2)	1.88^{C}
	MEAN	$2.14^{A,B}$	0.39^{A}	0.15^{B}	

Ave SEM=0.36; For like letters $p<0.05$.

Table 8. Sera IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 1 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	-0.06 (7)	0.57 (6)	0.29 (2)	0.27
	no	0.67 (2)	1.46 (1)	0.57 (1)	0.90
	MEAN	0.31	1.01	0.43	

Ave SEM=0.71

Table 9. Sera IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 7 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.61	1.08	0.76	0.82
	no	0.76 (1)	-0.69 (1)	0.76 (2)	0.28
	MEAN	0.69	0.19	0.76	

Ave SEM=0.99

Table 10. Sera IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 21 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	-0.08 (4)	0.54	0.37 (2)	0.28
	no	1.66 (1)	na	0.58 (2)	1.12
	MEAN	0.79	0.54	0.48	

Ave SEM=0.77

Table 11. Nasal IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 1 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.55 (4)	0.03 (4)	0.51 (2)	0.362
	no	na	0.01 (3)	-0.02 (1)	0.003
	MEAN	0.55	0.02	0.24	

Ave SEM=0.57

Table 12. Nasal IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 7 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	1.03 (3)	0.43 (6)	0.96 (4)	0.81
	no	0.87 (2)	0.75 (1)	1.10 (2)	0.91
	MEAN	0.95	0.59	1.03	

Ave SEM=0.60

Table 13. Nasal IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 14 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.02^{A}(5)	0.56^{B}(4)	0.32^{C} (4)	0.30
	no	-0.16^{A} (1)	$3.06^{A,B,C}$(2)	-0.11^{B} (3)	0.93
	MEAN	-0.07	1.81	0.10	

Ave SEM=1.09; $^{A,B}p<0.10$, $^{C}p<0.05$

Table 14. Nasal IgA titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 21 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	2.31 (4)	0.63 (4)	1.98 (2)	1.64
	no	-0.19 (1)	na	0.85 (2)	0.33
	MEAN	1.06	0.63	1.42	

Ave SEM=1.99

Table 15. Nasal IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 1 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	1.63 (4)	0.66 (4)	1.26 (2)	1.18
	no	na	0.36 (3)	0.60 (1)	0.48
	MEAN	1.63	0.51	0.93	

Ave SEM=0.81

Table 16. Nasal IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 7 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.49 (3)	0.14 (6)	0.16 (4)	0.26
	no	0.04 (1)	0.02 (1)	0.40 (2)	0.15
	MEAN	0.27	0.08	0.28	

Ave SEM=0.33

Table 17. Nasal IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 14 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.03[A] (5)	0.62[A] (5)	0.59 (4)	0.41
	no	0.54 (1)	0.54 (2)	0.44 (3)	0.51
	MEAN	0.28	0.48	0.51	

Ave SEM=0.34; [A]p<0.10

Table 18. Nasal IgG titers (log base 2 and corrected by subtraction of baseline titer) in gilts fed varying levels of HMB once daily, day 21 post-farrowing (titer (n)).

		HMB (gms per day)			
		0	2	10	MEAN
VACC	yes	0.21 (4)	0.32 (4)	0.16 (2)	0.23
	no	-0.09 (1)	na	0.57 (2)	0.24
	MEAN	0.06	0.32	0.36	

Ave SEM=0.31

Table 19. Sera anti-PMT IgG (log base 2), Day 7 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	9.92	8.24	9.08
	no	8.99	8.07	8.53
	MEAN	9.46	8.16	

Ave SEM = 0.76; p for treatment interaction = 0.62

Table 20. Sera anti-PMT IgG (log base 2), Day 21 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	9.14	9.13	9.13
	no	8.10	8.96	8.53
	MEAN	8.62	9.04	

Ave SEM = 0.42; p for treatment interaction = 0.30

Table 21. Sera anti-PMT IgG (log base 2), Day 35 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	9.23	8.54	8.88
	no	8.72	8.56	8.64
	MEAN	8.98	8.55	

Ave SEM = 0.49; p for treatment interaction = 0.58

Table 22. Sera anti-PMT IgG (log base 2), Day 70 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	9.58	8.72	9.15
	no	9.06	8.12	8.59
	MEAN	9.32	8.42	

Ave SEM = 0.34; p for treatment interaction = 0.90

Table 23. Sera anti-PMT IgG (log base 2), Day 98 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	10.57	9.64	10.1
	no	8.82	8.75	8.78
	MEAN	9.70	9.20	

Ave SEM = 0.75; p for treatment interaction = 0.45

Table 24. Sera anti-PMT IgG (log base 2), Day 124 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	9.11	10.08	9.60
	no	9.83	10.15	9.99
	MEAN	9.47	10.10	

Ave SEM = 0.36; p for treatment interaction = 0.39

Table 25. Sera anti-PMT IgA (log base 2), Day 7 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	3.54	3.79	3.66
	no	4.57	2.58	3.58
	MEAN	4.06	3.18	

Ave SEM = 1.73; p for treatment interaction = 0.54

Table 26. Sera anti-PMT IgA (log base 2), Day 21 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	4.10	3.47	3.78
	no	5.54	2.34	3.94
	MEAN	4.82	2.90	

Ave SEM = 0.92; p for treatment interaction = 0.26

Table 27. Sera anti-PMT IgA (log base 2), Day 35 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	2.81	1.30	2.06
	no	4.74	2.63	3.68
	MEAN	3.78	1.96	

Ave SEM = 3.07; p for treatment interaction = 0.94

Table 28. Sera anti-PMT IgA (log base 2), Day 70 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	2.19	2.90	2.54
	no	3.66	3.85	3.76
	MEAN	2.92	3.38	

Ave SEM = 1.64; p for treatment interaction = 0.88

Table 29. Sera anti-PMT IgA (log base 2), Day 124 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	3.47	2.77	3.12
	no	3.92	5.60	4.76
	MEAN	3.70	4.18	

Ave SEM = 0.53; p for treatment interaction = 0.23

Table 30. Nasal anti-PMT IgG (log base 2), Day 35 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	1.37	-0.20[A]	0.58
	no	3.35[A]	1.36	2.35
	MEAN	2.36	0.58	

Ave SEM = 1.34; p for interaction = 0.88; [A]p = 0.08

Table 31. Nasal anti-PMT IgA (log base 2), Day 35 post-experimental toxigenic *P. multocida* exposure in 6 wk old pigs

		Exp Exposure		
		yes	no	MEAN
VACC	yes	-0.11	0.45	0.34
	no	1.91	2.43	2.17
		0.90	1.44	

Ave SEM = 1.16; p for interaction = 0.98